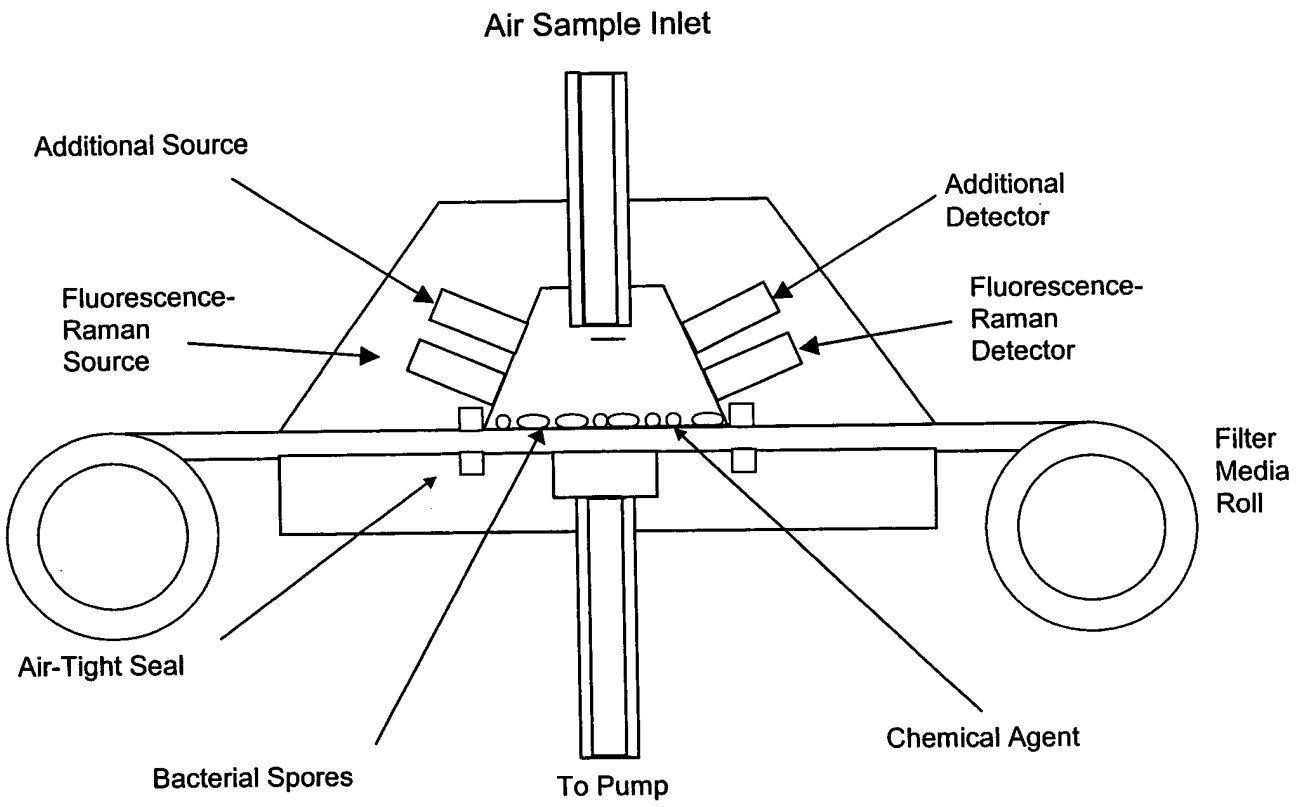


The Integrated Detector Sample Cell

Figure 1

Dispersive Raman Spectra of Anthrax Simulants

Using a ChemIcon FALCON™ Raman Chemical Imaging Microscope

Brightfield Transmittance Image

- Bacteria deposited on microscope slide from aqueous suspension
- Microscope slide background spectrum subtracted from sample spectra

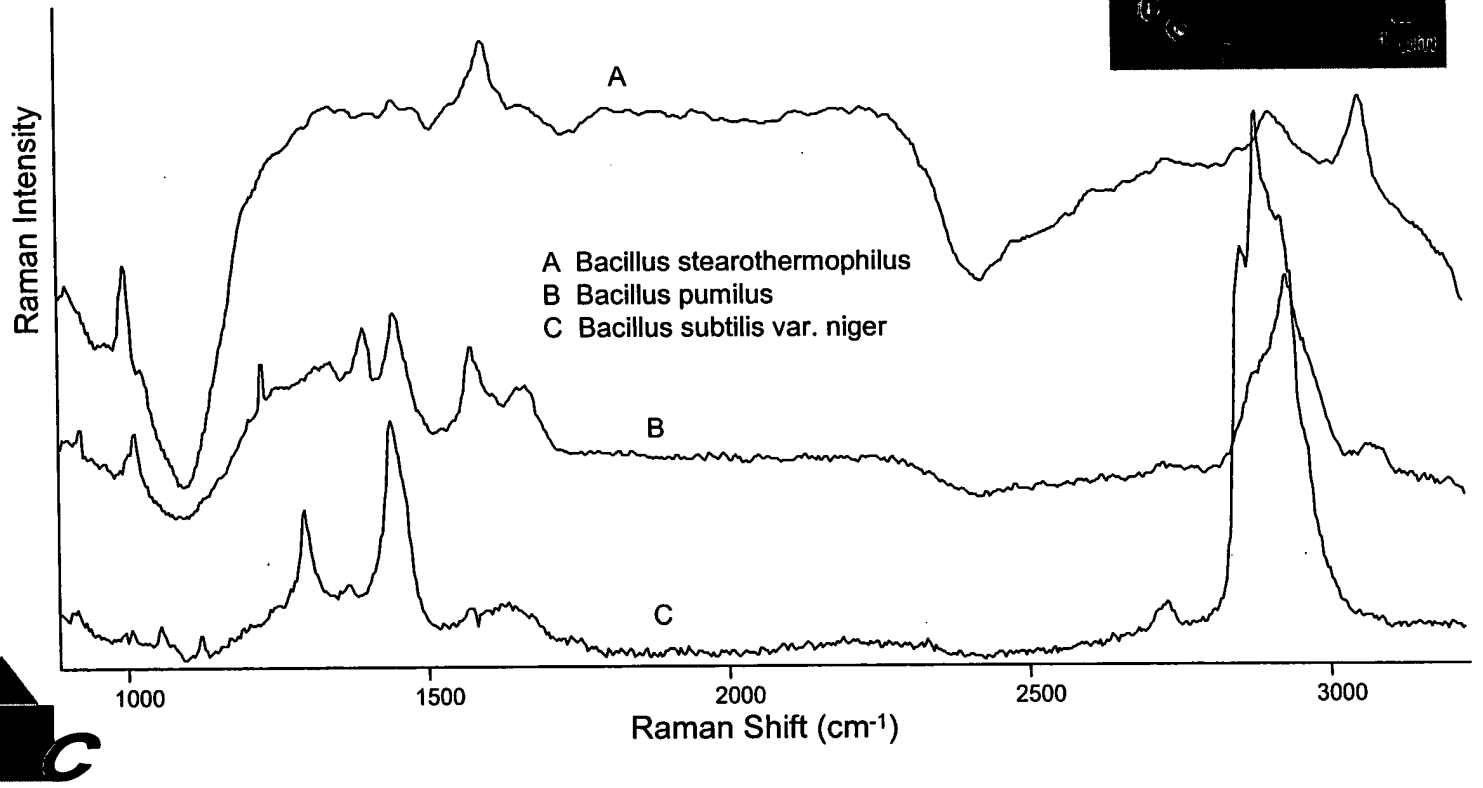
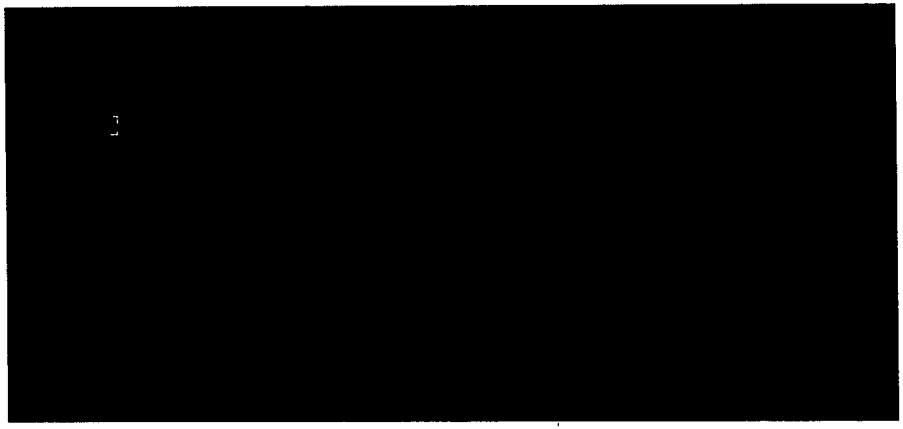


Figure 2

Fluorescence Chemical Imaging of *Bacillus Pumilus* and *Bacillus Subtilis* Mixture



Bacillus subtilis

Bacillus pumilus

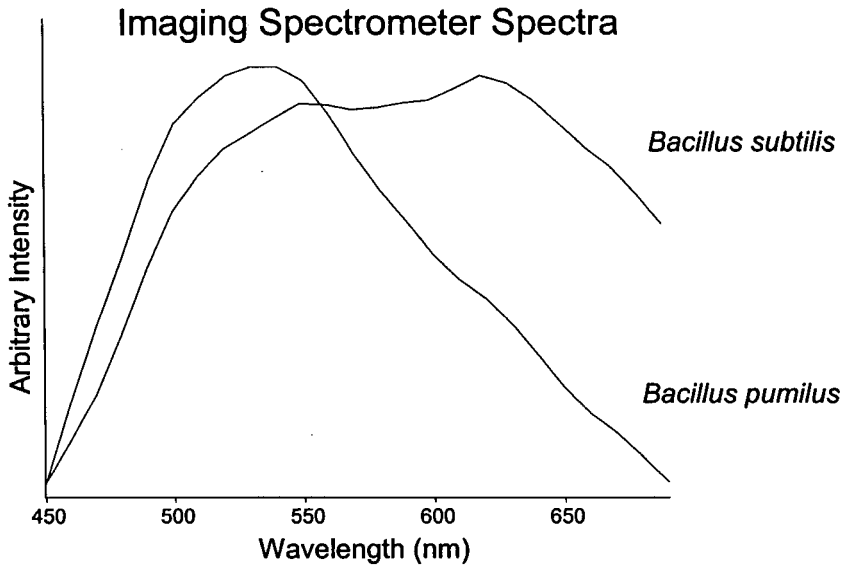


Figure 3

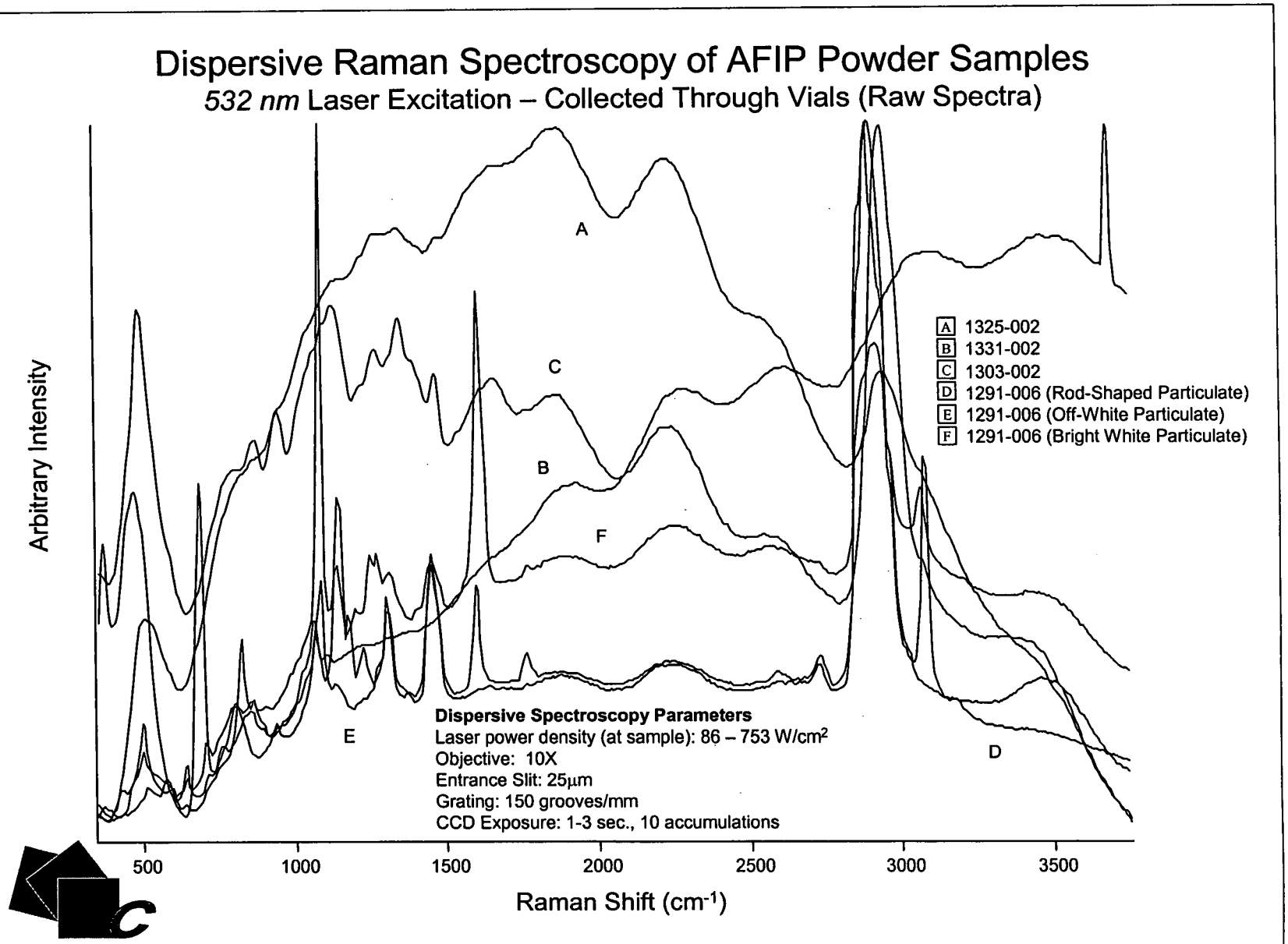

Dispersive Raman Spectroscopy of AFIP Powder Samples
532 nm Laser Excitation – Collected Through Vials (Raw Spectra)
Arbitrary Intensity
A
B
C
D
E
F
A 1325-002
B 1331-002
C 1303-002
D 1291-006 (Rod-Shaped Particulate)
E 1291-006 (Off-White Particulate)
F 1291-006 (Bright White Particulate)
Dispersive Spectroscopy Parameters
Laser power density (at sample): 86 – 753 W/cm2
Objective: 10X
Entrance Slit: 25µm
Grating: 150 grooves/mm
CCD Exposure: 1-3 sec., 10 accumulations
500
1000
1500
2000
2500
3000
3500
Raman Shift (cm-1)

Figure 4A

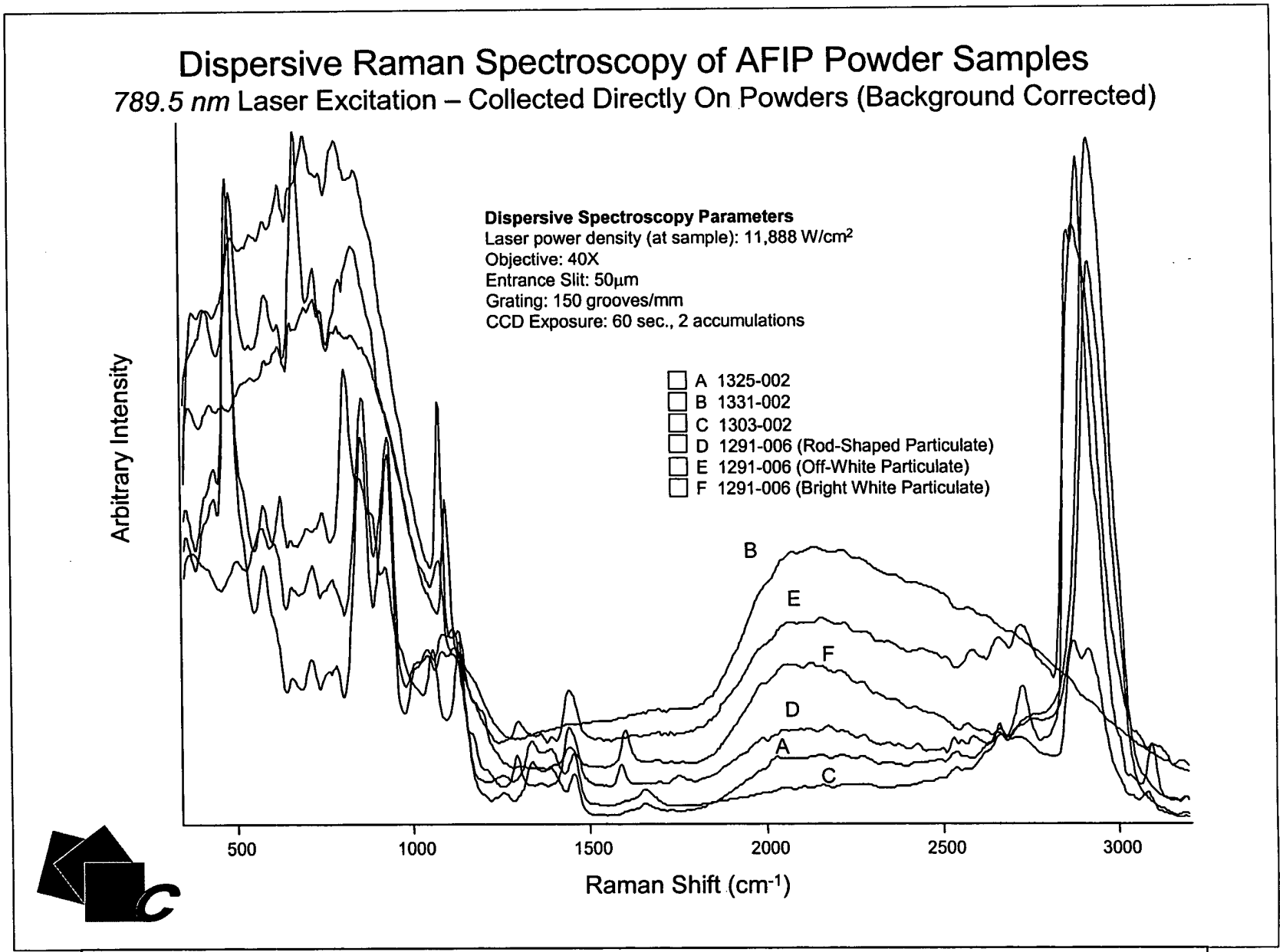

Dispersive Raman Spectroscopy of AFIP Powder Samples
789.5 nm Laser Excitation – Collected Directly On Powders (Background Corrected)
Dispersive Spectroscopy Parameters
Laser power density (at sample): 11,888 W/cm2
Objective: 40X
Entrance Slit: 50µm
Grating: 150 grooves/mm
CCD Exposure: 60 sec., 2 accumulations
A 1325-002
B 1331-002
C 1303-002
D 1291-006 (Rod-Shaped Particulate)
E 1291-006 (Off-White Particulate)
F 1291-006 (Bright White Particulate)
Arbitrary Intensity
B
E
F
D
A
C
500
1000
1500
2000
2500
3000
Raman Shift (cm-1)

Figure 4B

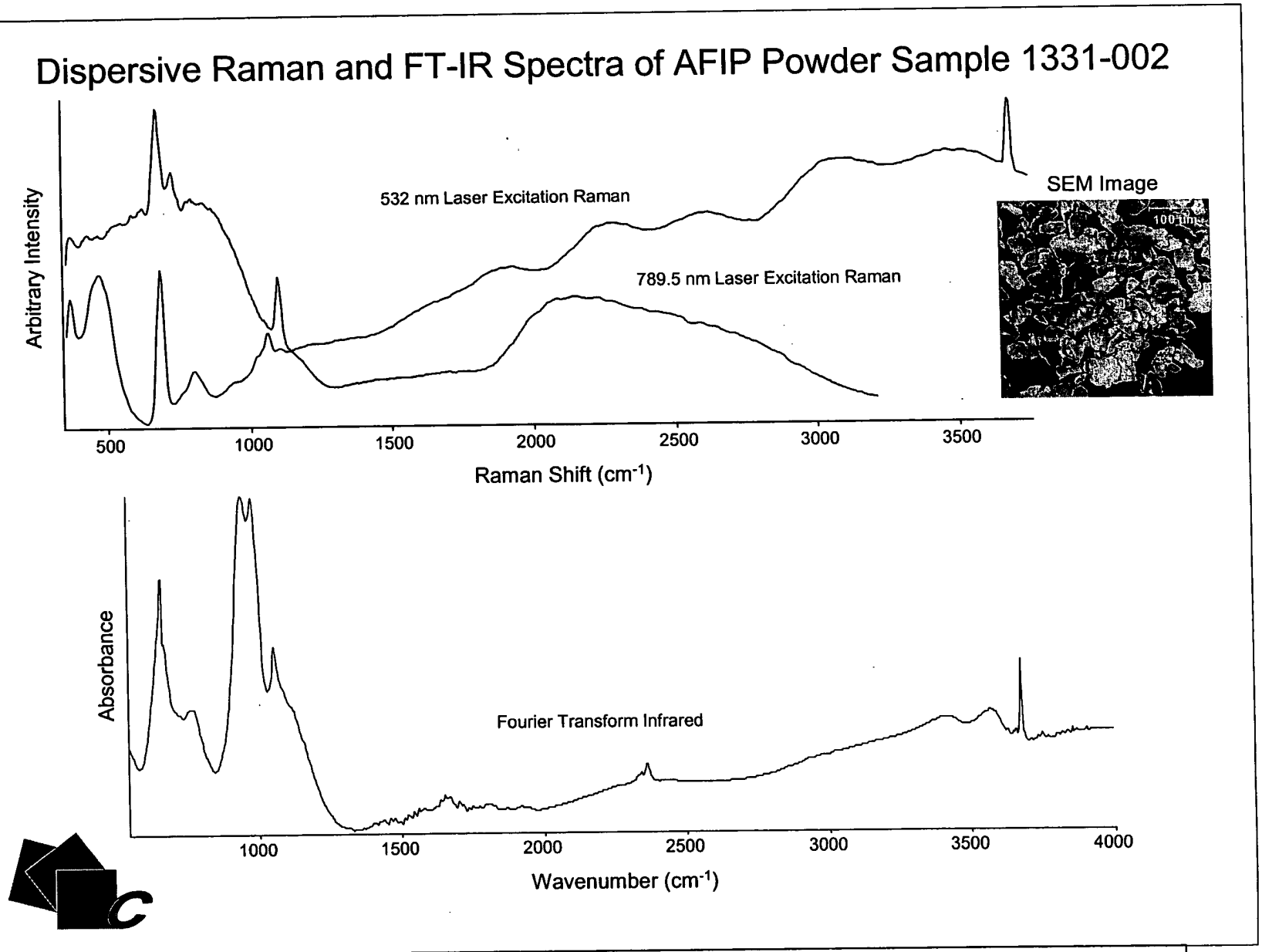
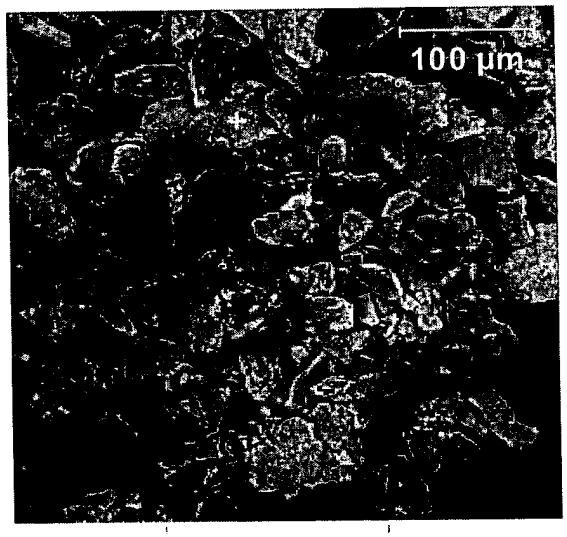

Dispersive Raman and FT-IR Spectra of AFIP Powder Sample 1331-002
Arbitrary Intensity
532 nm Laser Excitation Raman
789.5 nm Laser Excitation Raman
SEM Image
100 µm
500
1000
1500
2000
2500
3000
3500
Raman Shift (cm-1)
Absorbance
Fourier Transform Infrared
1000
1500
2000
2500
3000
3500
4000
Wavenumber (cm-1)

Figure 4C

SEM/EDS of AFIP Powder Sample 1331-002

Backscattered Electron Image



Energy Dispersive Spectrum

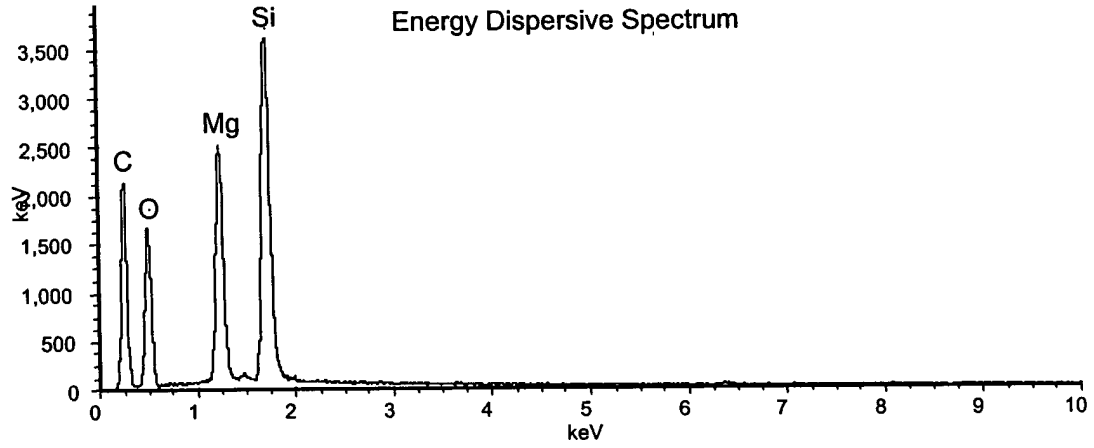


Figure 4D

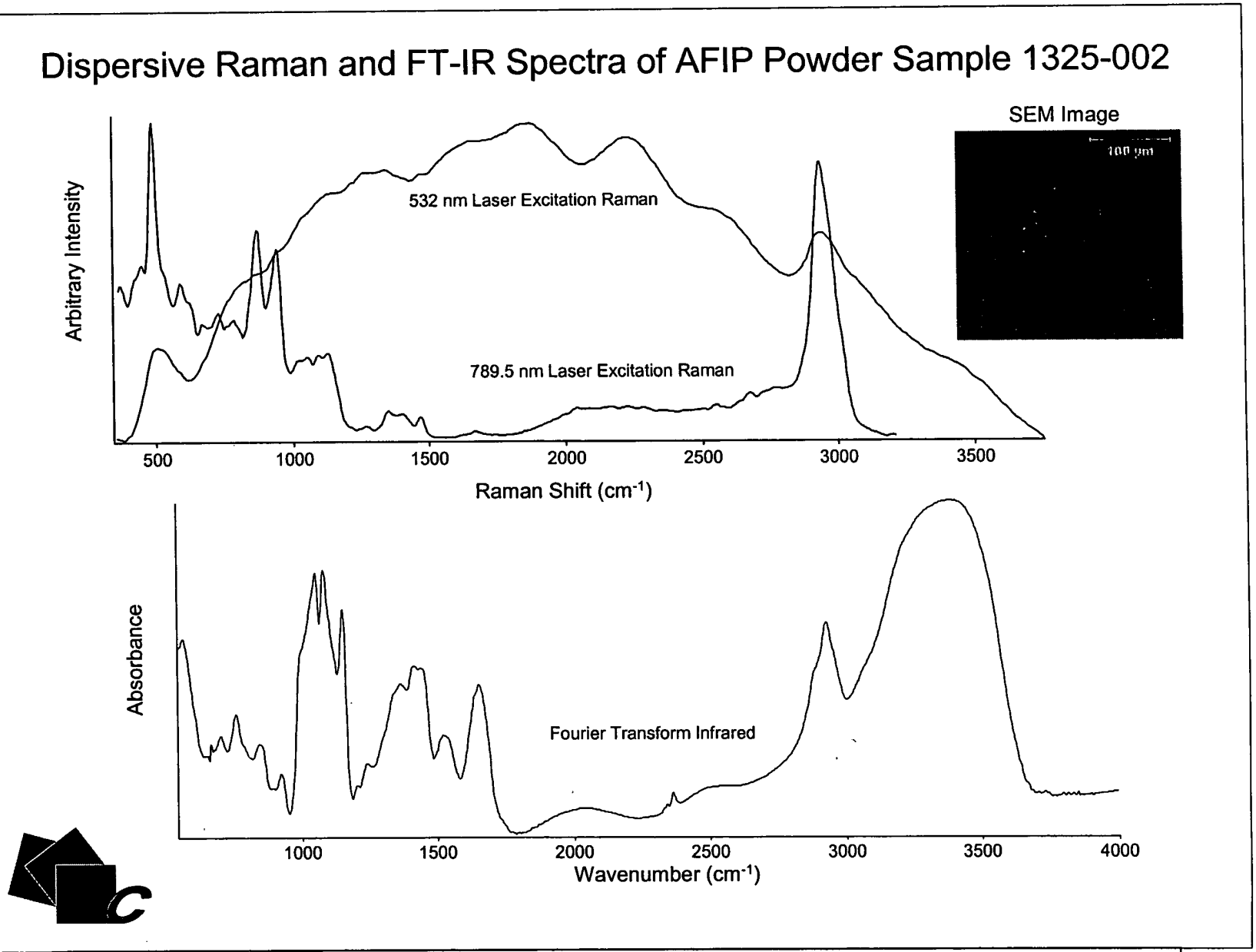
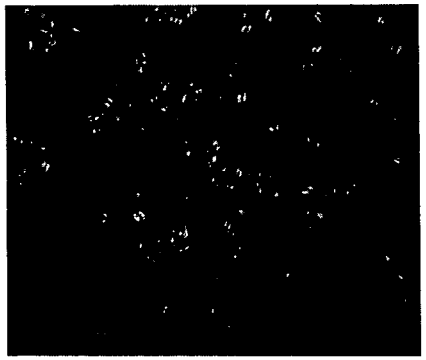

Dispersive Raman and FT-IR Spectra of AFIP Powder Sample 1325-002
SEM Image
100 µm
Arbitrary Intensity
532 nm Laser Excitation Raman
789.5 nm Laser Excitation Raman
500
1000
1500
2000
2500
3000
3500
Raman Shift (cm-1)
Absorbance
Fourier Transform Infrared
1000
1500
2000
2500
3000
3500
4000
Wavenumber (cm-1)

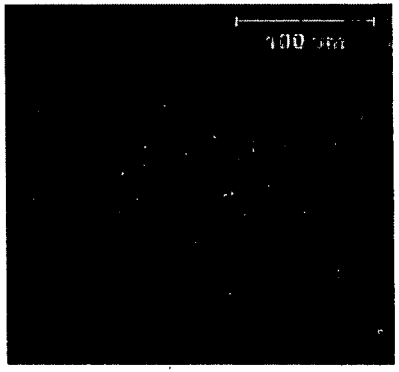
Figure 4E

SEM/EDS of AFIP Powder Sample 1325-002

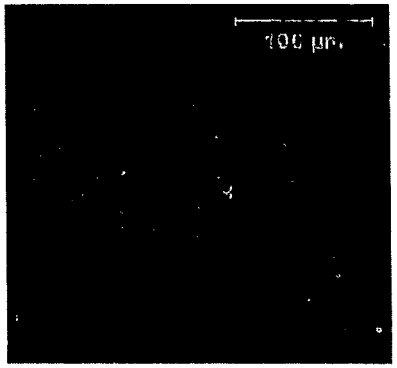
Polarized Light Image



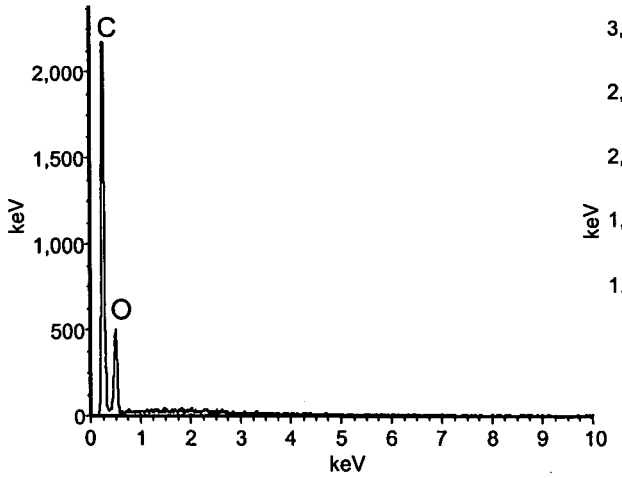
Backscattered Electron Image



Backscattered Electron Image



Energy Dispersive Spectrum



Energy Dispersive Spectrum

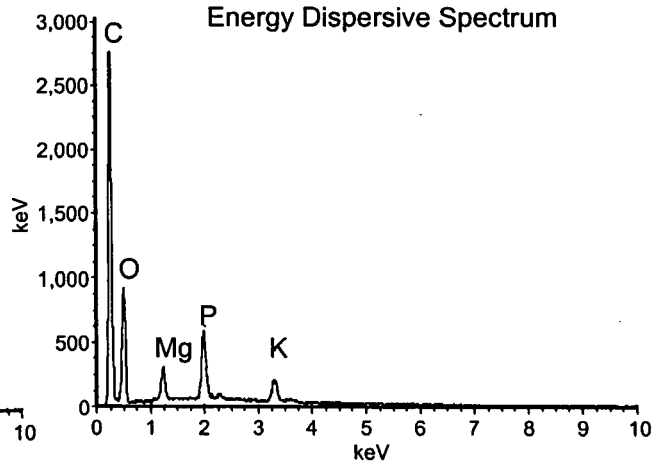


Figure 4F

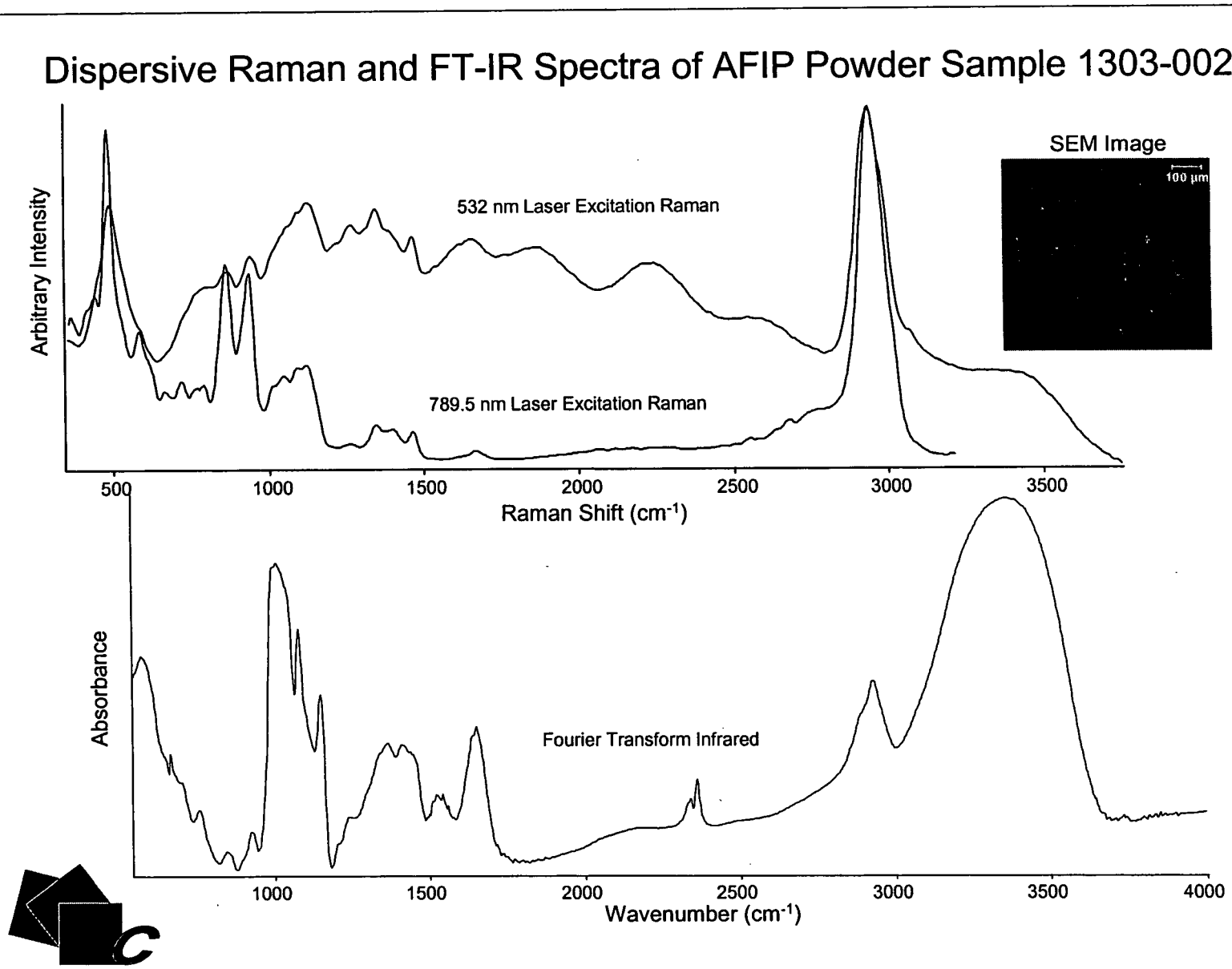
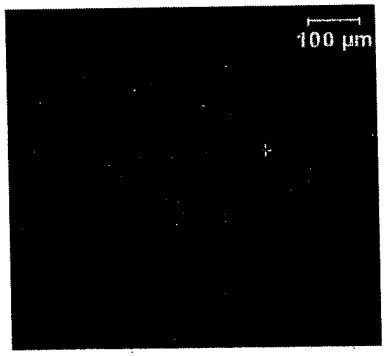

Dispersive Raman and FT-IR Spectra of AFIP Powder Sample 1303-002
SEM Image
100 µm
Arbitrary Intensity
532 nm Laser Excitation Raman
789.5 nm Laser Excitation Raman
500
1000
1500
2000
2500
3000
3500
Raman Shift (cm-1)
Absorbance
Fourier Transform Infrared
1000
1500
2000
2500
3000
3500
4000
Wavenumber (cm-1)

Figure 4G

SEM/EDS of AFIP Powder Sample 1303-002

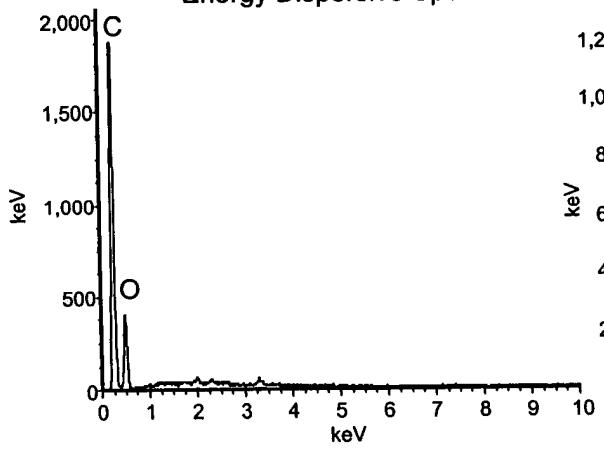
Polarized Light Image

Backscattered Electron Image



Backscattered Electron Image

Energy Dispersive Spectrum



Energy Dispersive Spectrum

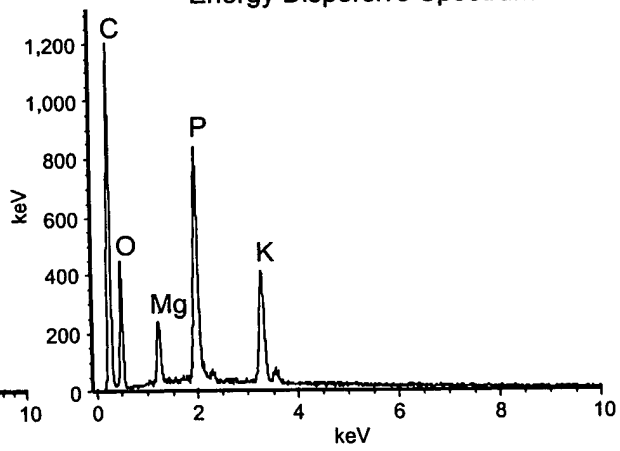


Figure 4H

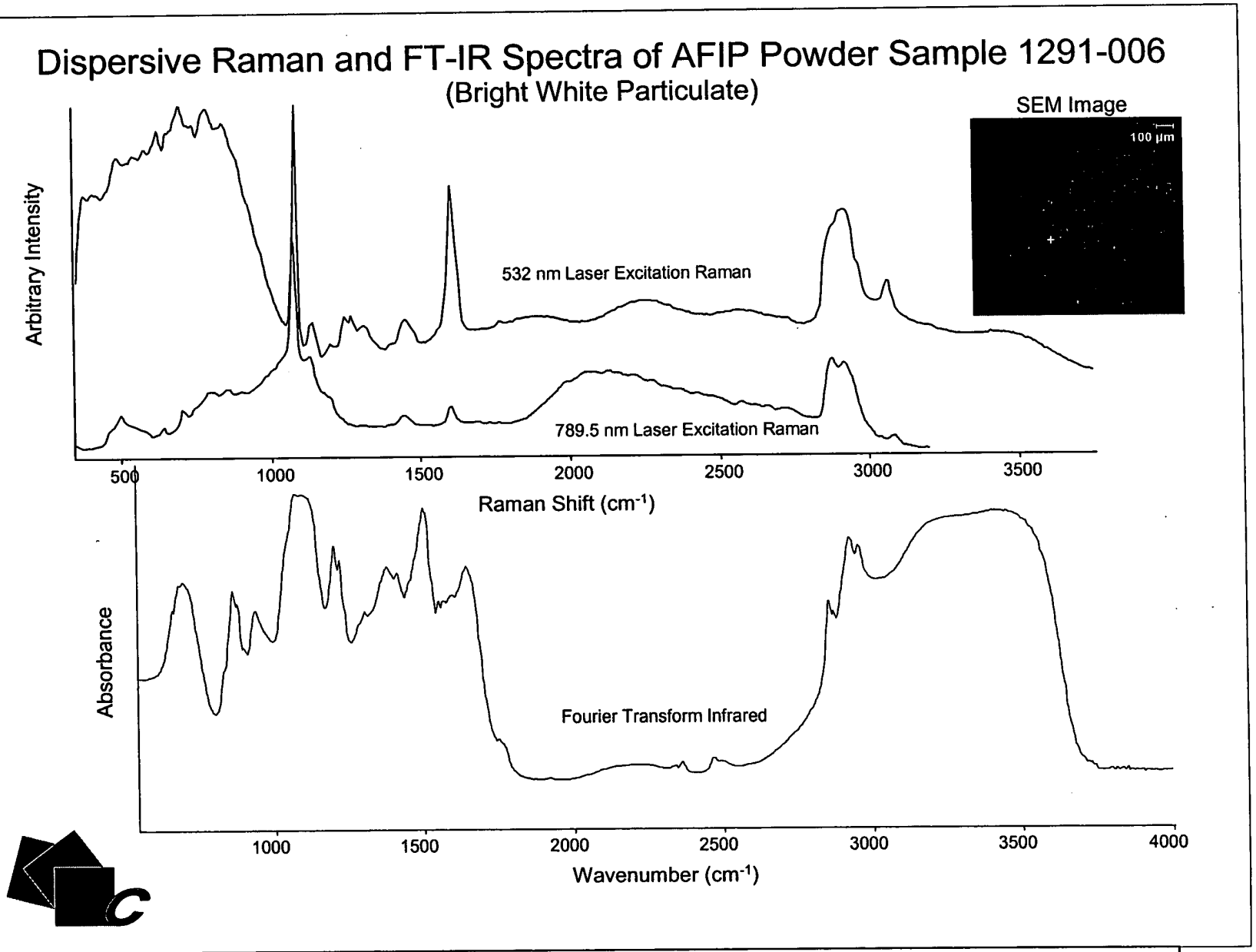
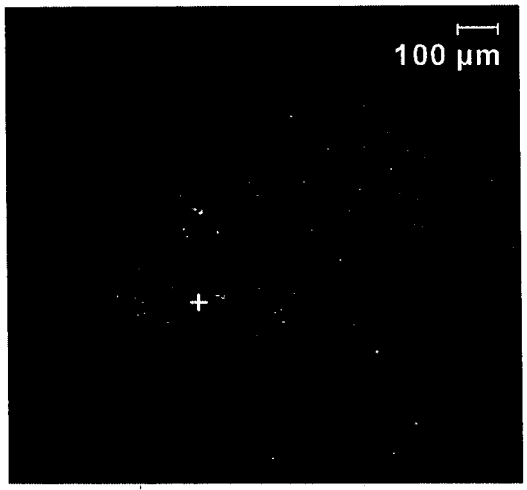

Dispersive Raman and FT-IR Spectra of AFIP Powder Sample 1291-006
(Bright White Particulate)
SEM Image
100 µm
Arbitrary Intensity
532 nm Laser Excitation Raman
789.5 nm Laser Excitation Raman
500
1000
1500
2000
2500
3000
3500
Raman Shift (cm-1)
Absorbance
Fourier Transform Infrared
1000
1500
2000
2500
3000
3500
4000
Wavenumber (cm-1)

Figure 4I

SEM/EDS of AFIP Powder Sample 1291-006
(Bright White Particulate)

Backscattered Electron Image



Energy Dispersive Spectrum

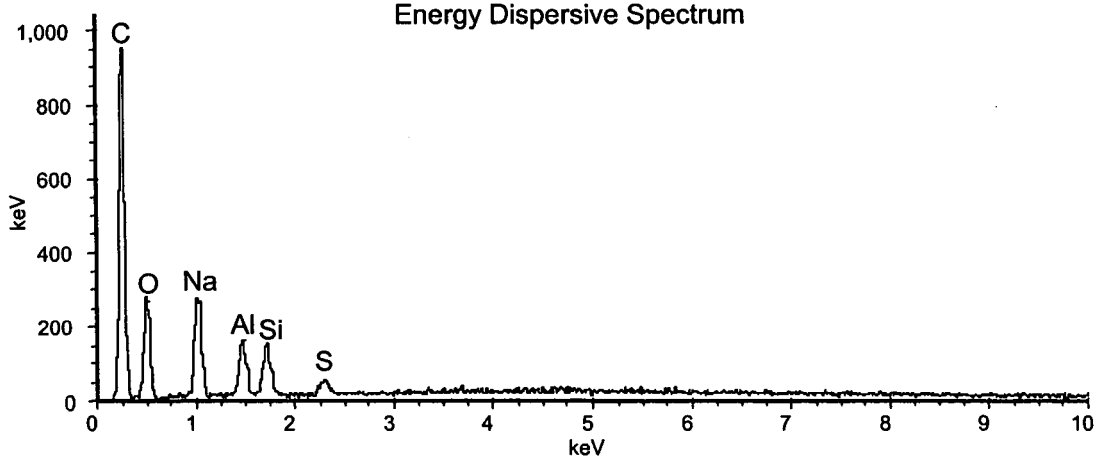


Figure 4J

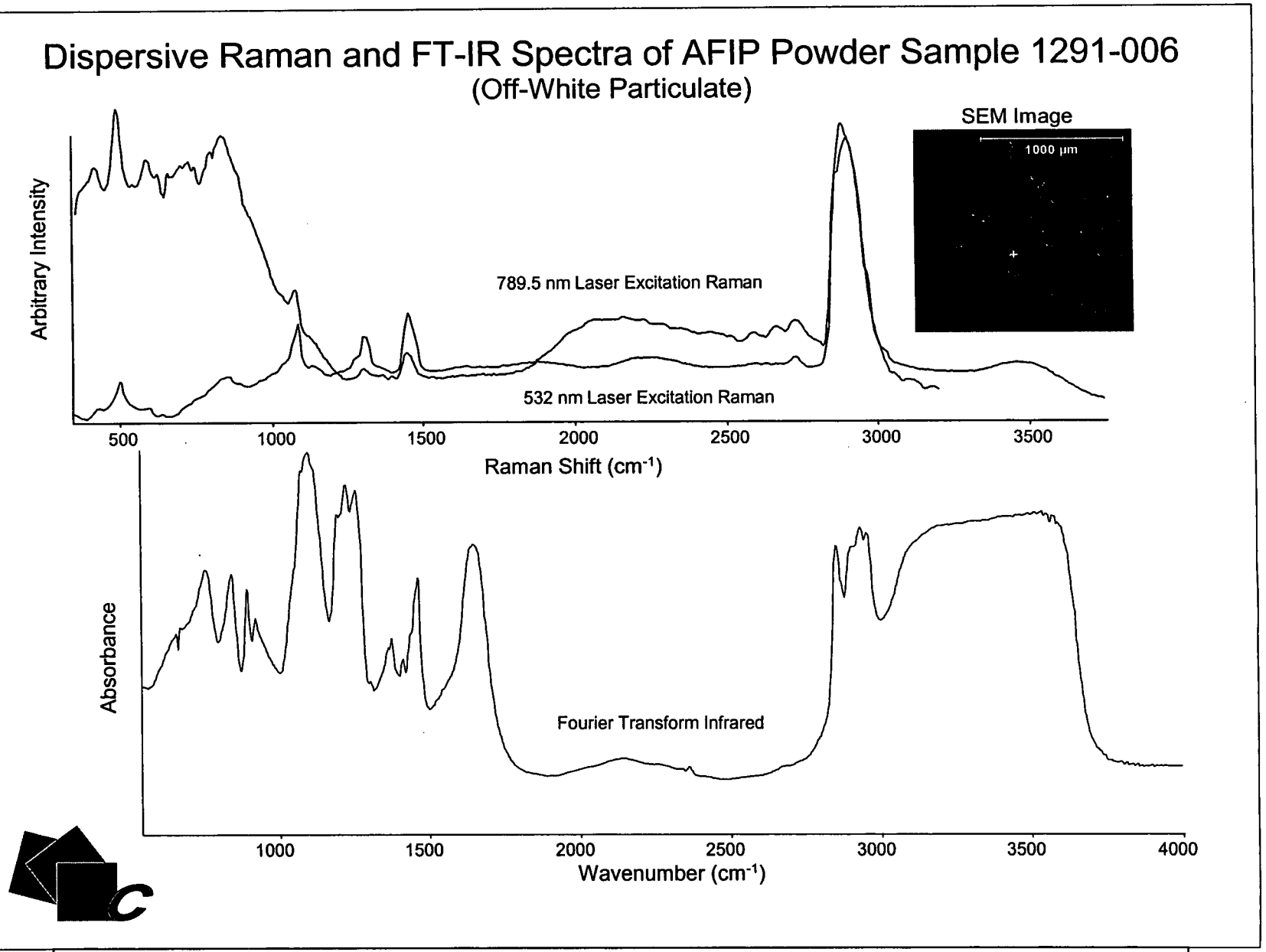
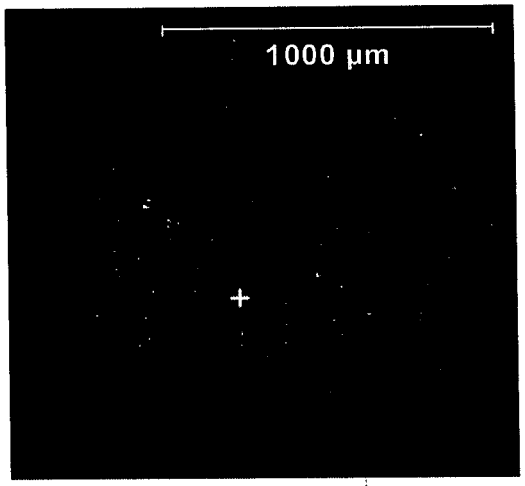

Dispersive Raman and FT-IR Spectra of AFIP Powder Sample 1291-006
(Off-White Particulate)
SEM Image
1000 µm
Arbitrary Intensity
789.5 nm Laser Excitation Raman
532 nm Laser Excitation Raman
500
1000
1500
2000
2500
3000
3500
Raman Shift (cm-1)
Absorbance
Fourier Transform Infrared
1000
1500
2000
2500
3000
3500
4000
Wavenumber (cm-1)

Figure 4K

SEM/EDS of AFIP Powder Sample 1291-006

(Off-White Particulate)

Backscattered Electron Image



Energy Dispersive Spectrum

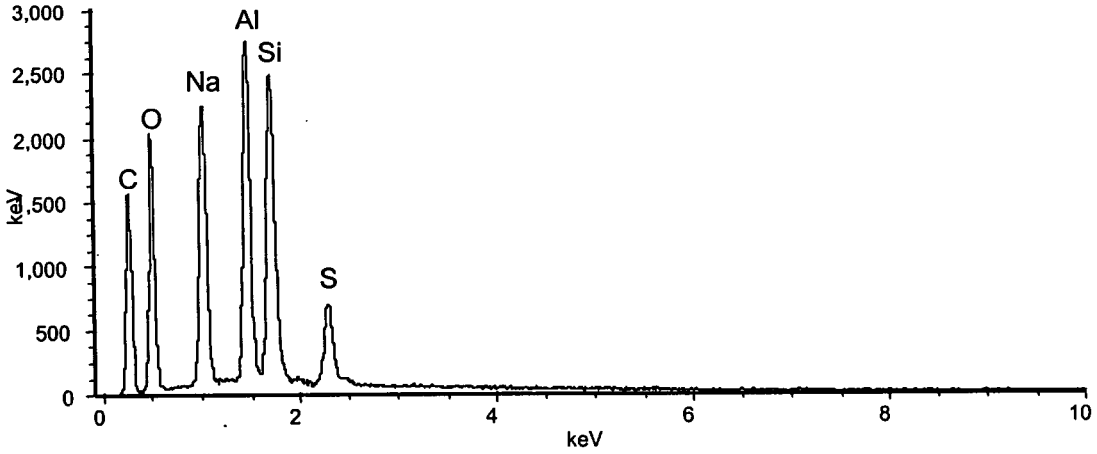


Figure 4L

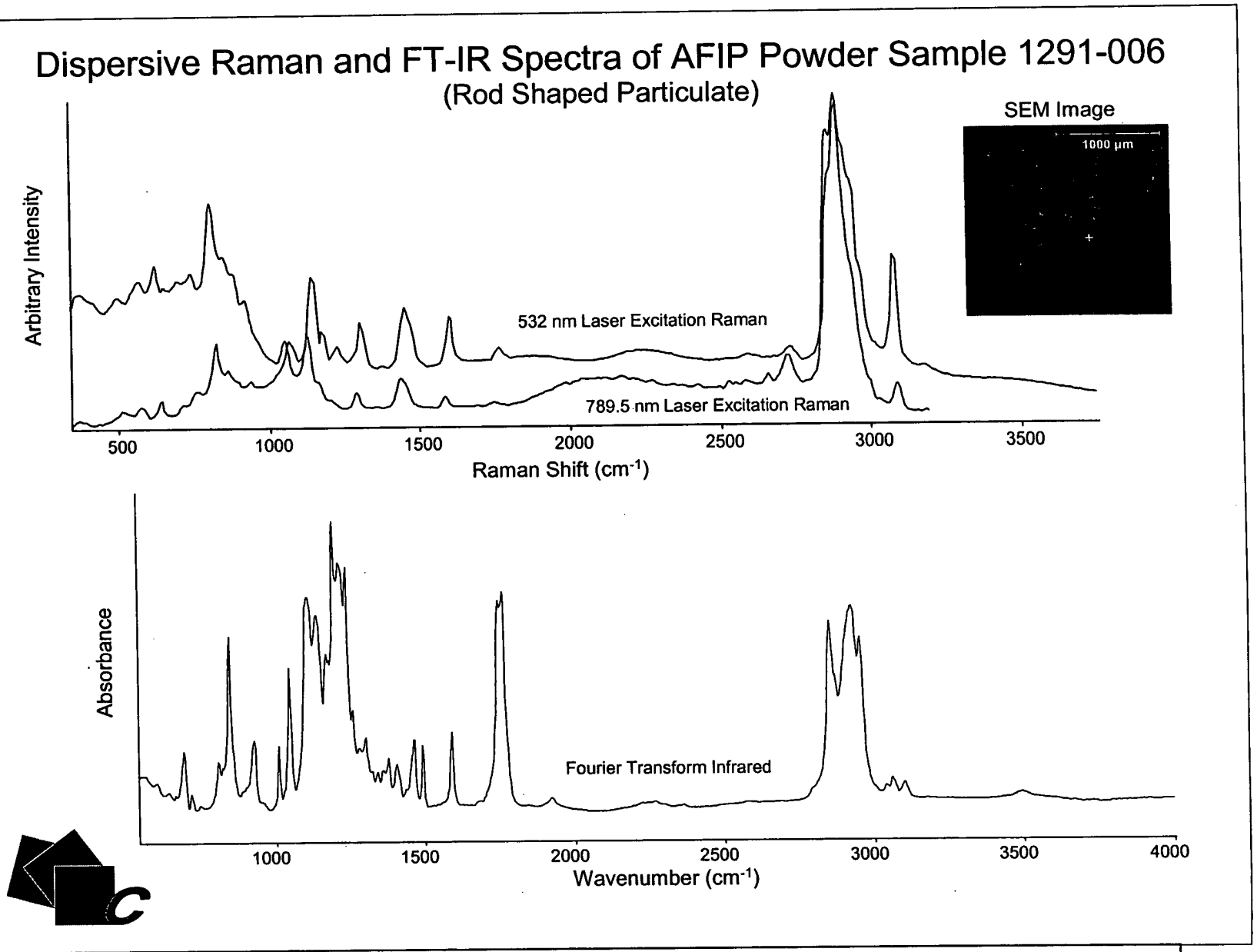
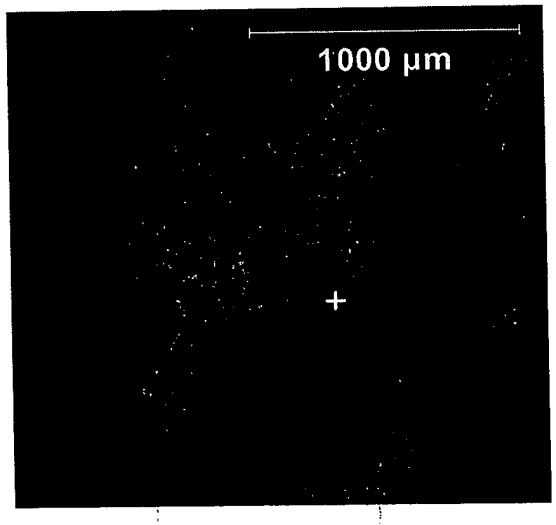

Dispersive Raman and FT-IR Spectra of AFIP Powder Sample 1291-006
(Rod Shaped Particulate)
SEM Image
1000 µm
Arbitrary Intensity
532 nm Laser Excitation Raman
789.5 nm Laser Excitation Raman
500
1000
1500
2000
2500
3000
3500
Raman Shift (cm-1)
Absorbance
Fourier Transform Infrared
1000
1500
2000
2500
3000
3500
4000
Wavenumber (cm-1)

Figure 4M

SEM/EDS of AFIP Powder Sample 1291-006
(Rod-Shaped Particulate)

Backscattered Electron Image



Energy Dispersive Spectrum

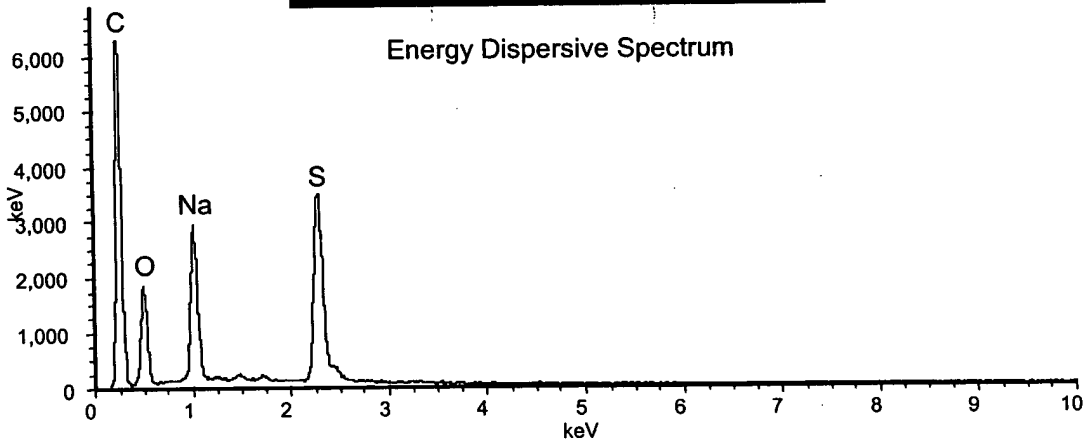


Figure 4N

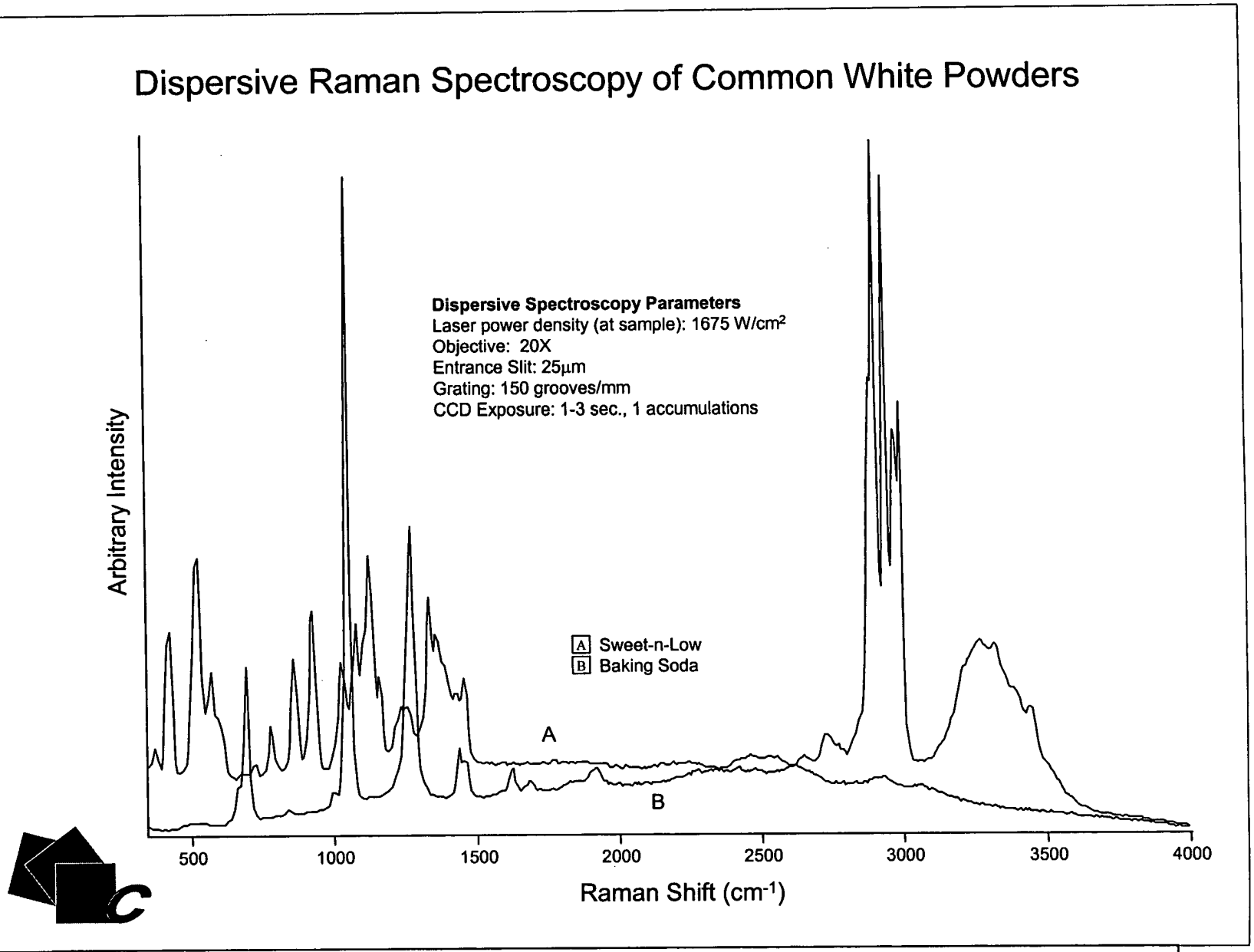

Dispersive Raman Spectroscopy of Common White Powders
Dispersive Spectroscopy Parameters
Laser power density (at sample): 1675 W/cm²
Objective: 20X
Entrance Slit: 25µm
Grating: 150 grooves/mm
CCD Exposure: 1-3 sec., 1 accumulations
A Sweet-n-Low
B Baking Soda
A
B
Arbitrary Intensity
500
1000
1500
2000
2500
3000
3500
4000
Raman Shift (cm-1)

Figure 4O

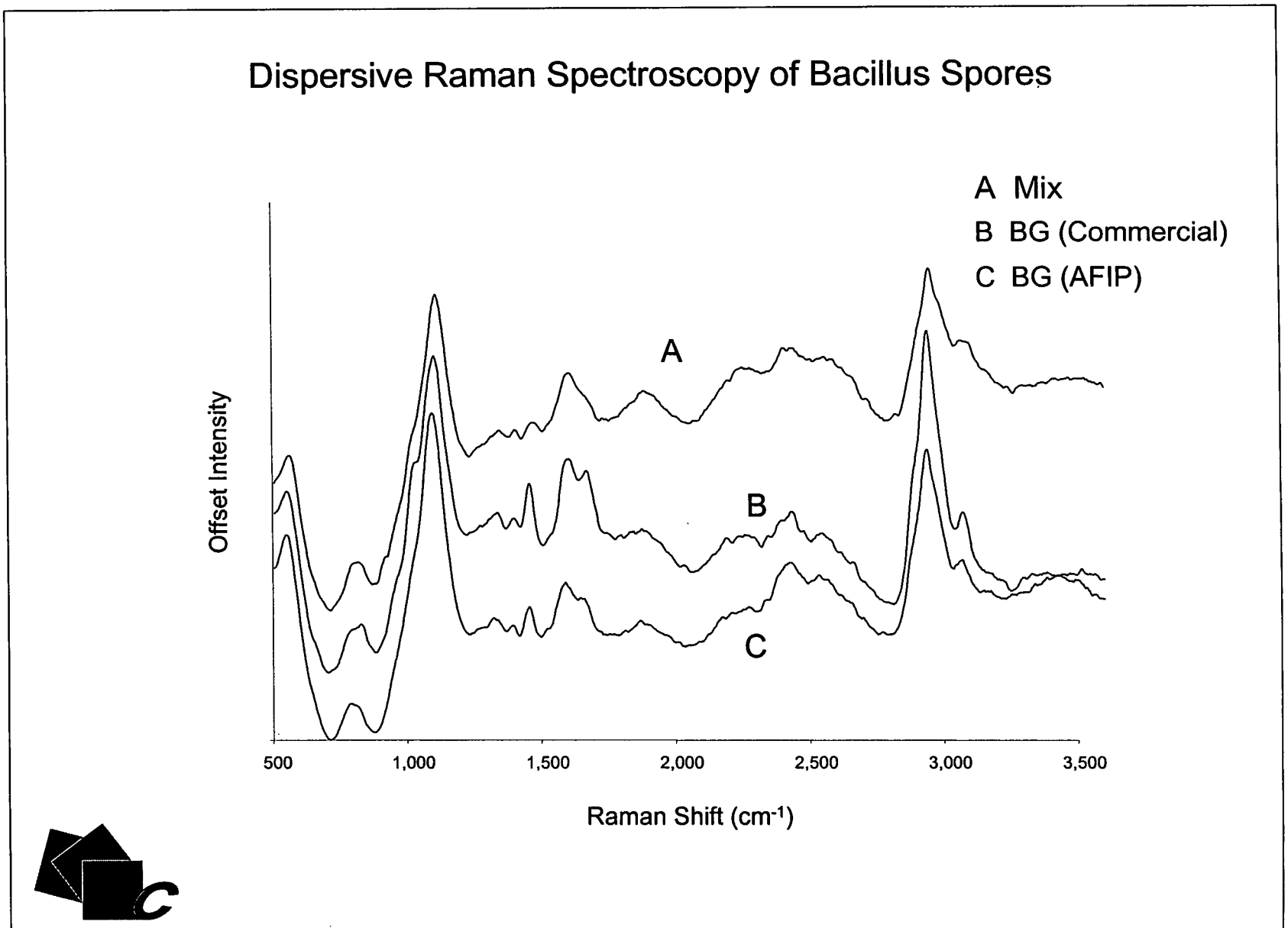
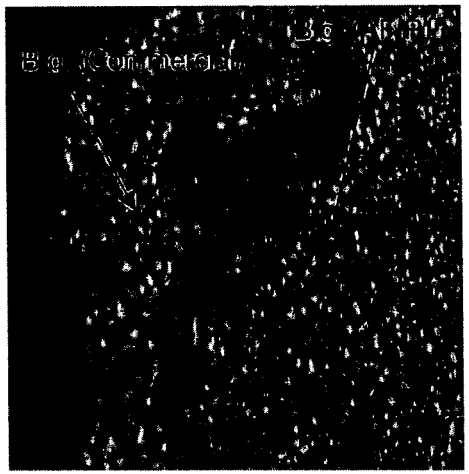
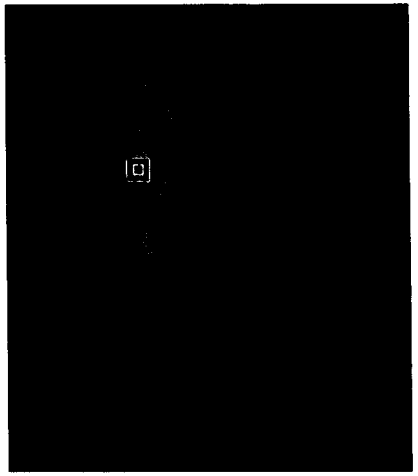

Dispersive Raman Spectroscopy of Bacillus Spores
A Mix
B BG (Commercial)
C BG (AFIP)
A
B
C
Offset Intensity
500
1,000
1,500
2,000
2,500
3,000
3,500
Raman Shift (cm-1)

Figure 4P

Raman Chemical Imaging of Bacillus Subtillus Spore Mixture

BG (AFIP) and BG (Commercial)





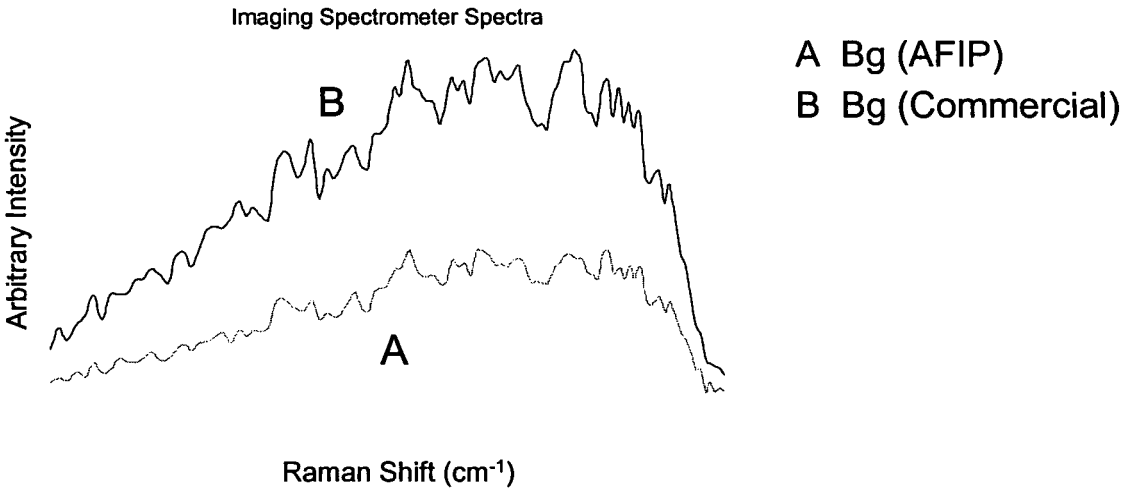


Figure 4Q

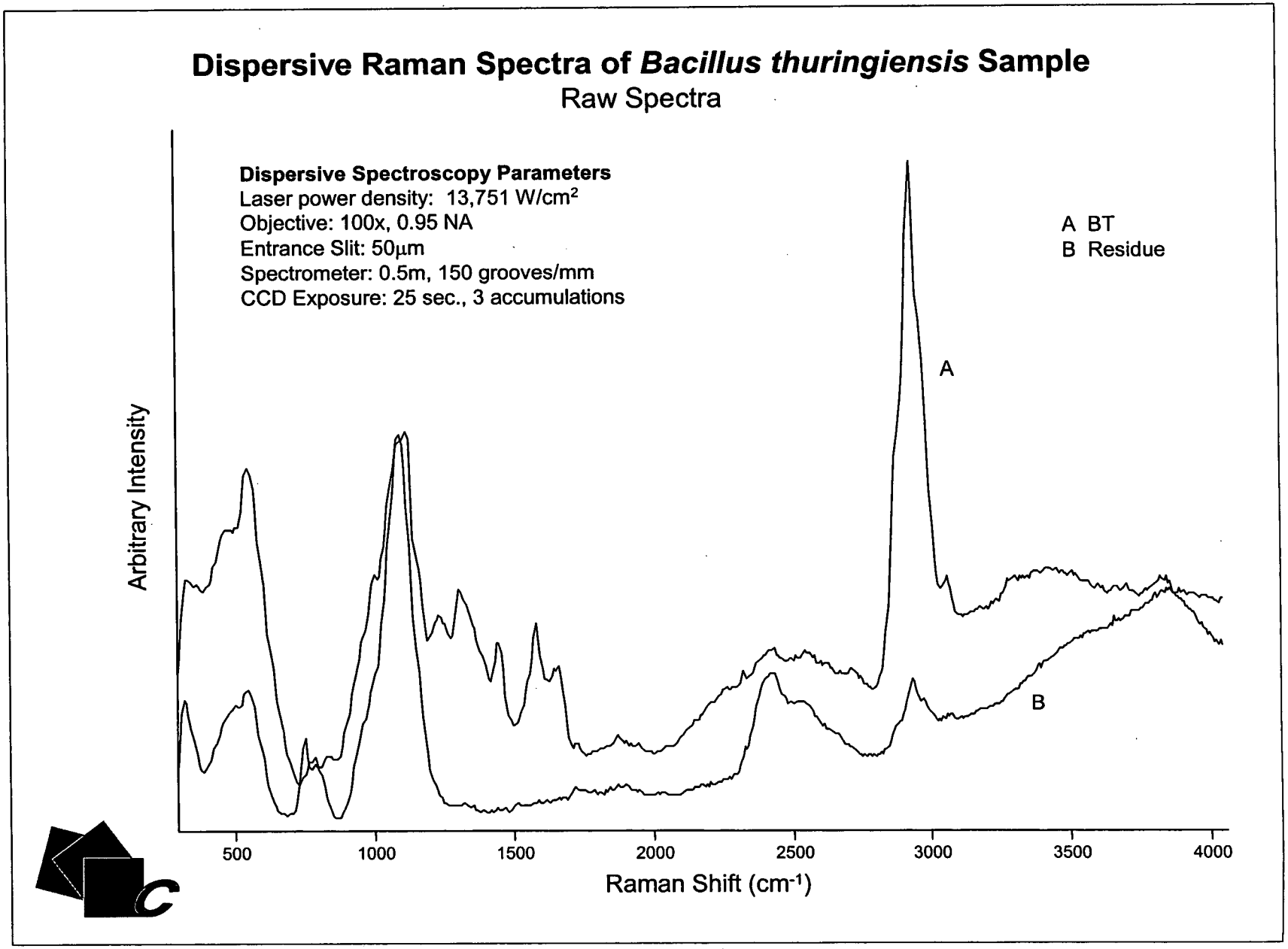

Dispersive Raman Spectra of *Bacillus thuringiensis* Sample
Raw Spectra
Dispersive Spectroscopy Parameters
Laser power density: 13,751 W/cm²
Objective: 100x, 0.95 NA
Entrance Slit: 50µm
Spectrometer: 0.5m, 150 grooves/mm
CCD Exposure: 25 sec., 3 accumulations
A BT
B Residue
A
B
Arbitrary Intensity
500
1000
1500
2000
2500
3000
3500
4000
Raman Shift (cm⁻¹)

Figure 5A

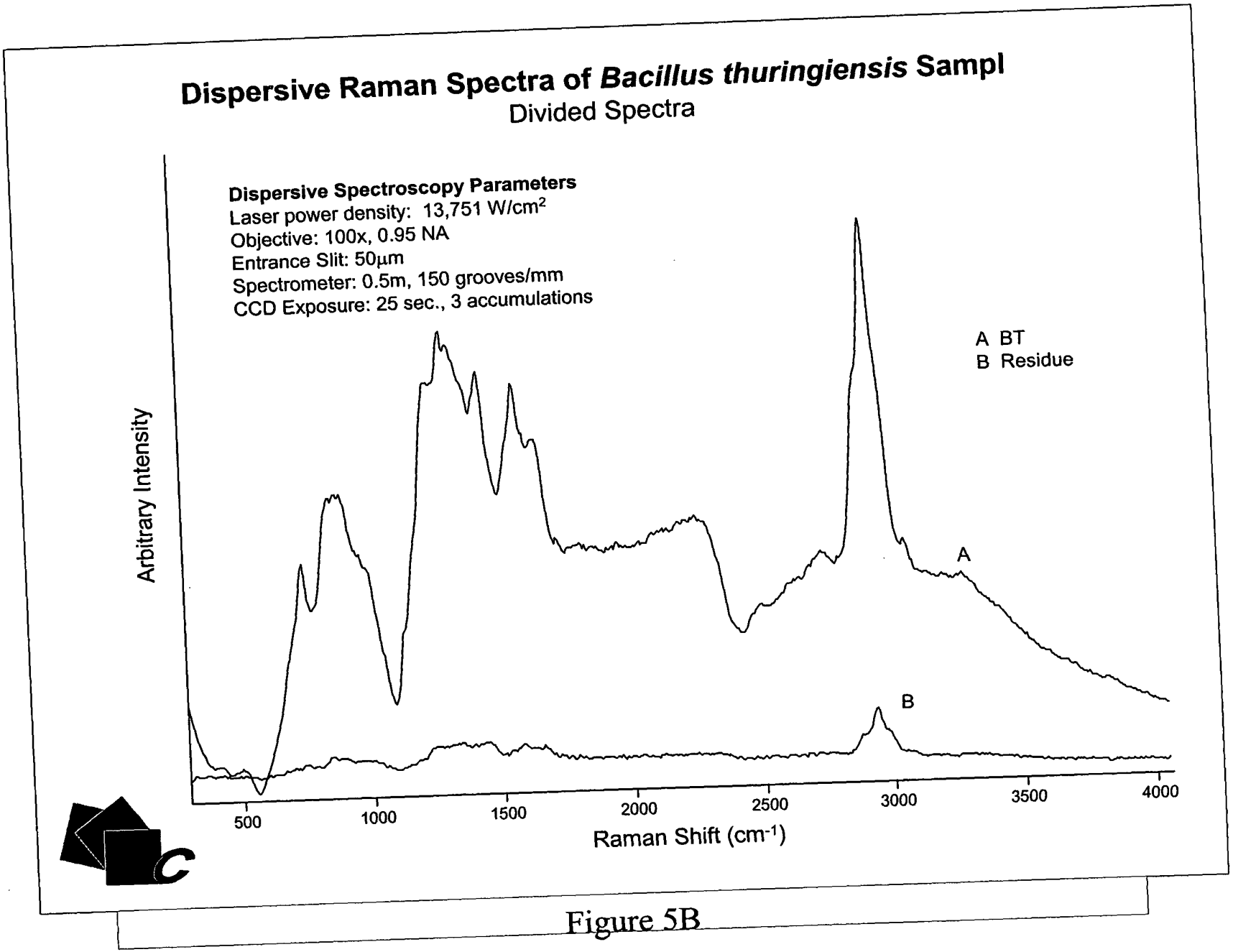

Dispersive Raman Spectra of Bacillus thuringiensis Sampl
Divided Spectra
Dispersive Spectroscopy Parameters
Laser power density: 13,751 W/cm2
Objective: 100x, 0.95 NA
Entrance Slit: 50µm
Spectrometer: 0.5m, 150 grooves/mm
CCD Exposure: 25 sec., 3 accumulations
A BT
B Residue
Arbitrary Intensity
A
B
500
1000
1500
2000
2500
3000
3500
4000
Raman Shift (cm-1)

Figure 5B

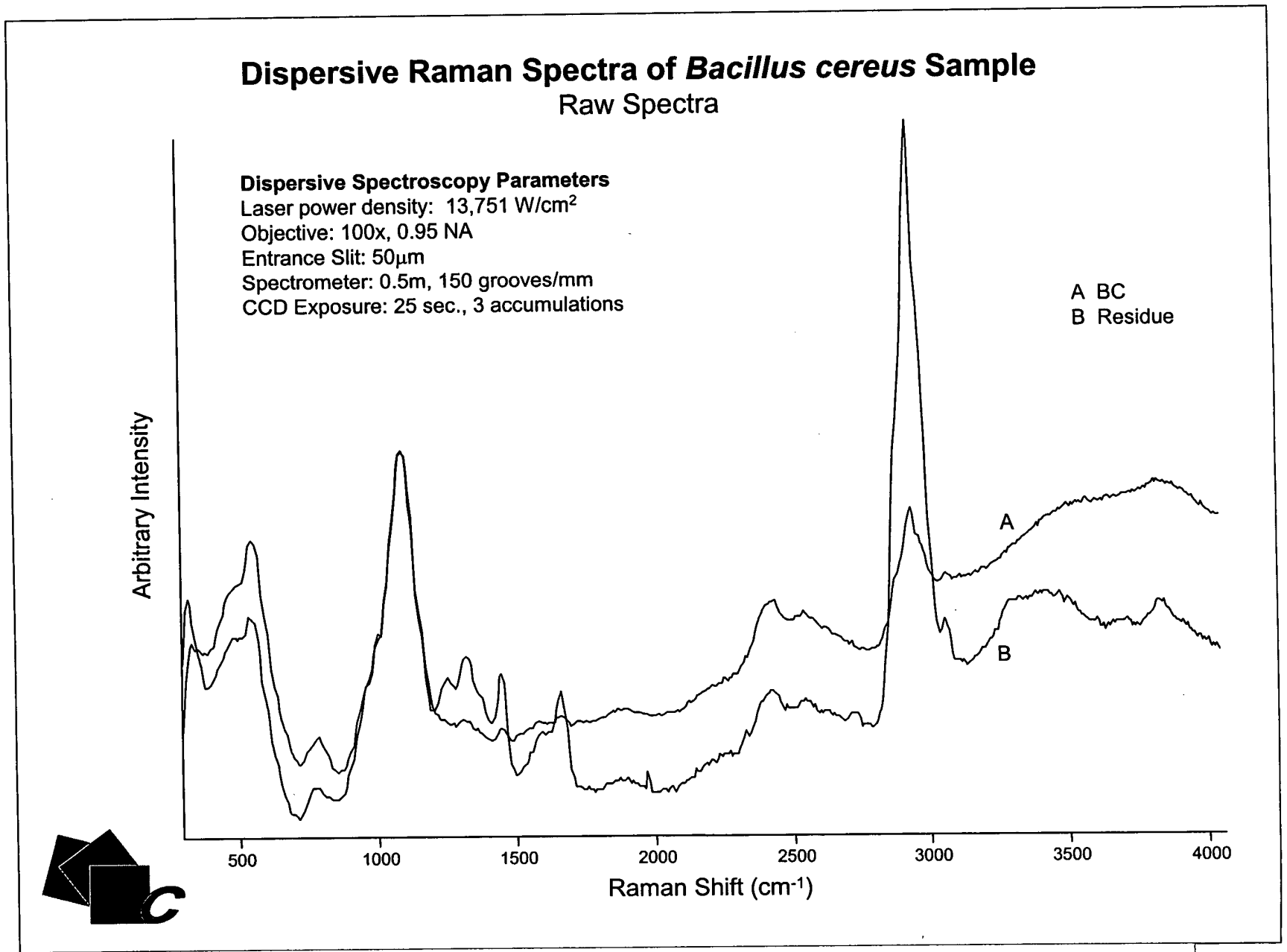

Dispersive Raman Spectra of *Bacillus cereus* Sample
Raw Spectra
Dispersive Spectroscopy Parameters
Laser power density: 13,751 W/cm²
Objective: 100x, 0.95 NA
Entrance Slit: 50µm
Spectrometer: 0.5m, 150 grooves/mm
CCD Exposure: 25 sec., 3 accumulations
A BC
B Residue
Arbitrary Intensity
A
B
500
1000
1500
2000
2500
3000
3500
4000
Raman Shift (cm⁻¹)

Figure 5C

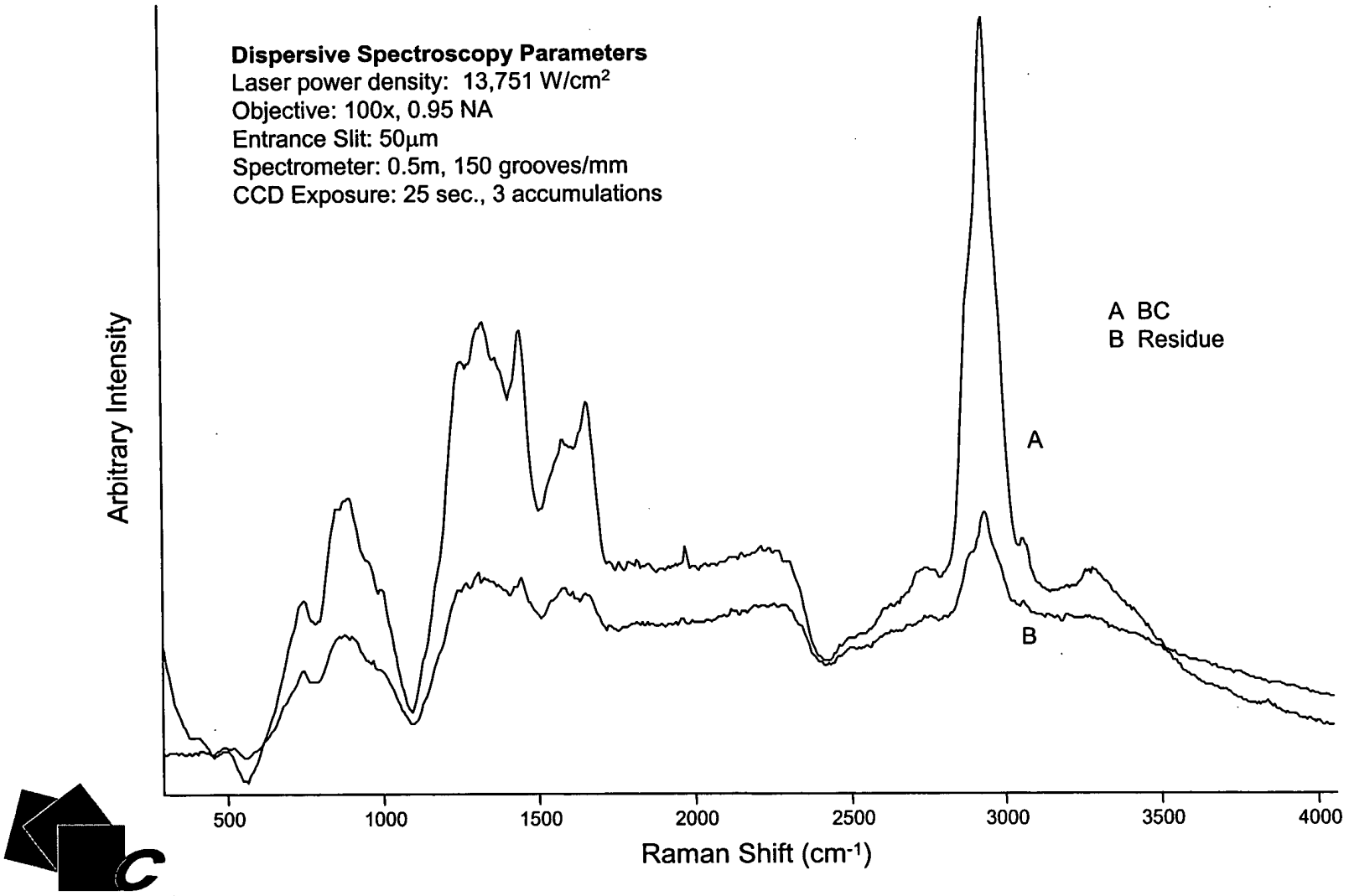

Dispersive Raman Spectra of *Bacillus cereus* Sample
Divided Spectra
Dispersive Spectroscopy Parameters
Laser power density: 13,751 W/cm²
Objective: 100x, 0.95 NA
Entrance Slit: 50µm
Spectrometer: 0.5m, 150 grooves/mm
CCD Exposure: 25 sec., 3 accumulations
A BC
B Residue
A
B
Arbitrary Intensity
500
1000
1500
2000
2500
3000
3500
4000
Raman Shift (cm⁻¹)

Figure 5D

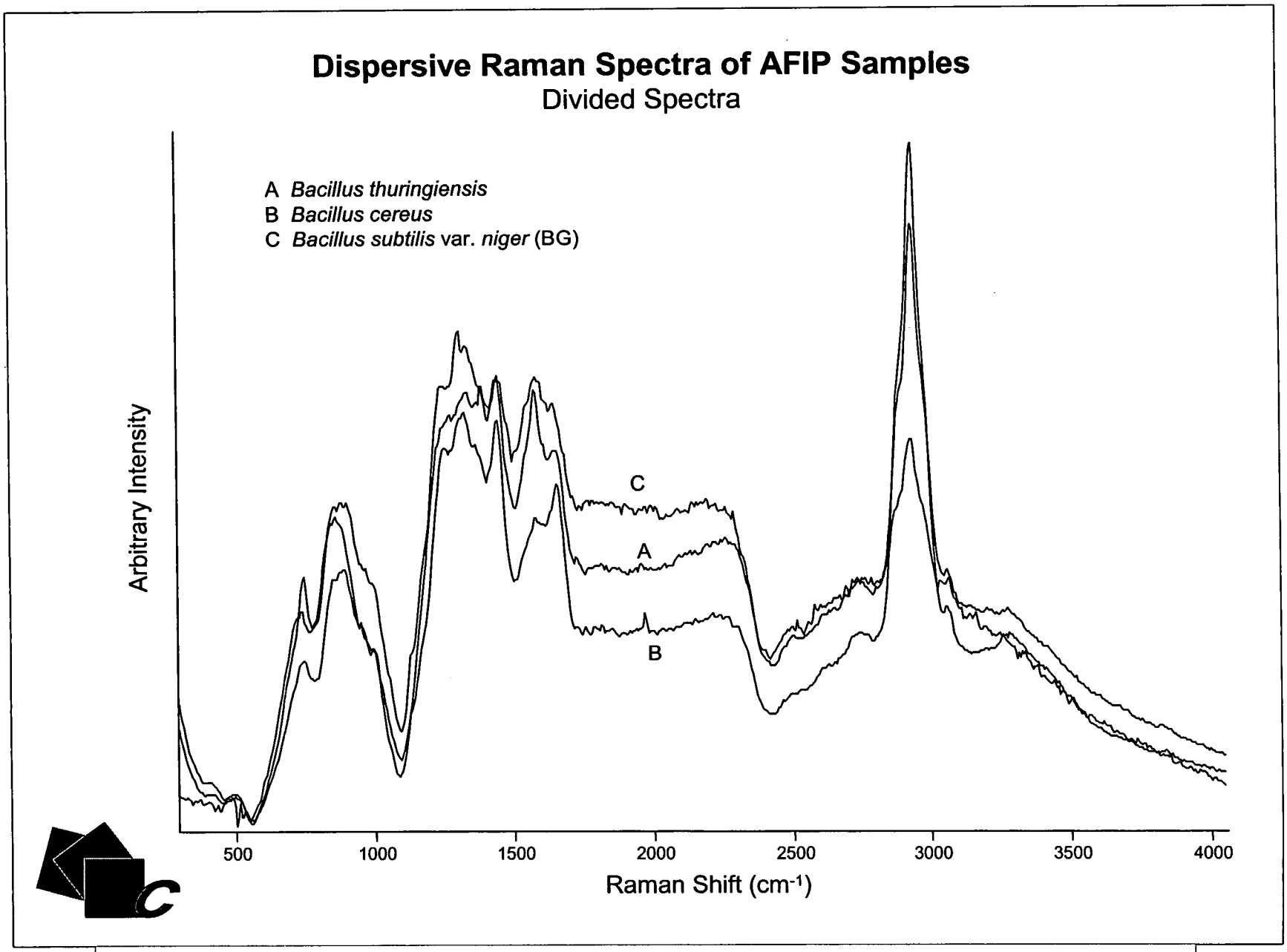

Dispersive Raman Spectra of AFIP Samples
Divided Spectra
A Bacillus thuringiensis
B Bacillus cereus
C Bacillus subtilis var. niger (BG)
Arbitrary Intensity
C
A
B
500
1000
1500
2000
2500
3000
3500
4000
Raman Shift (cm-1)

Figure 5E

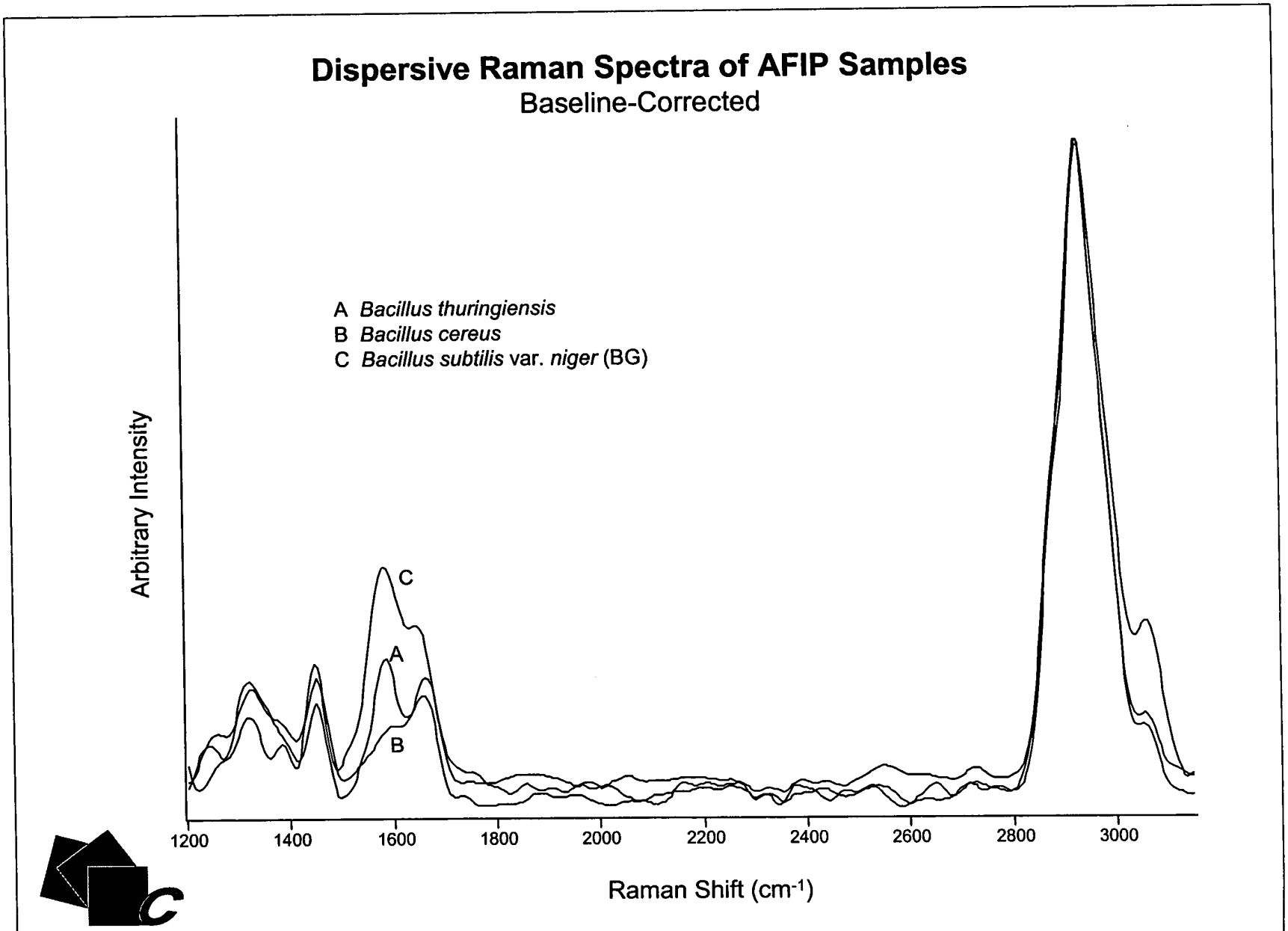
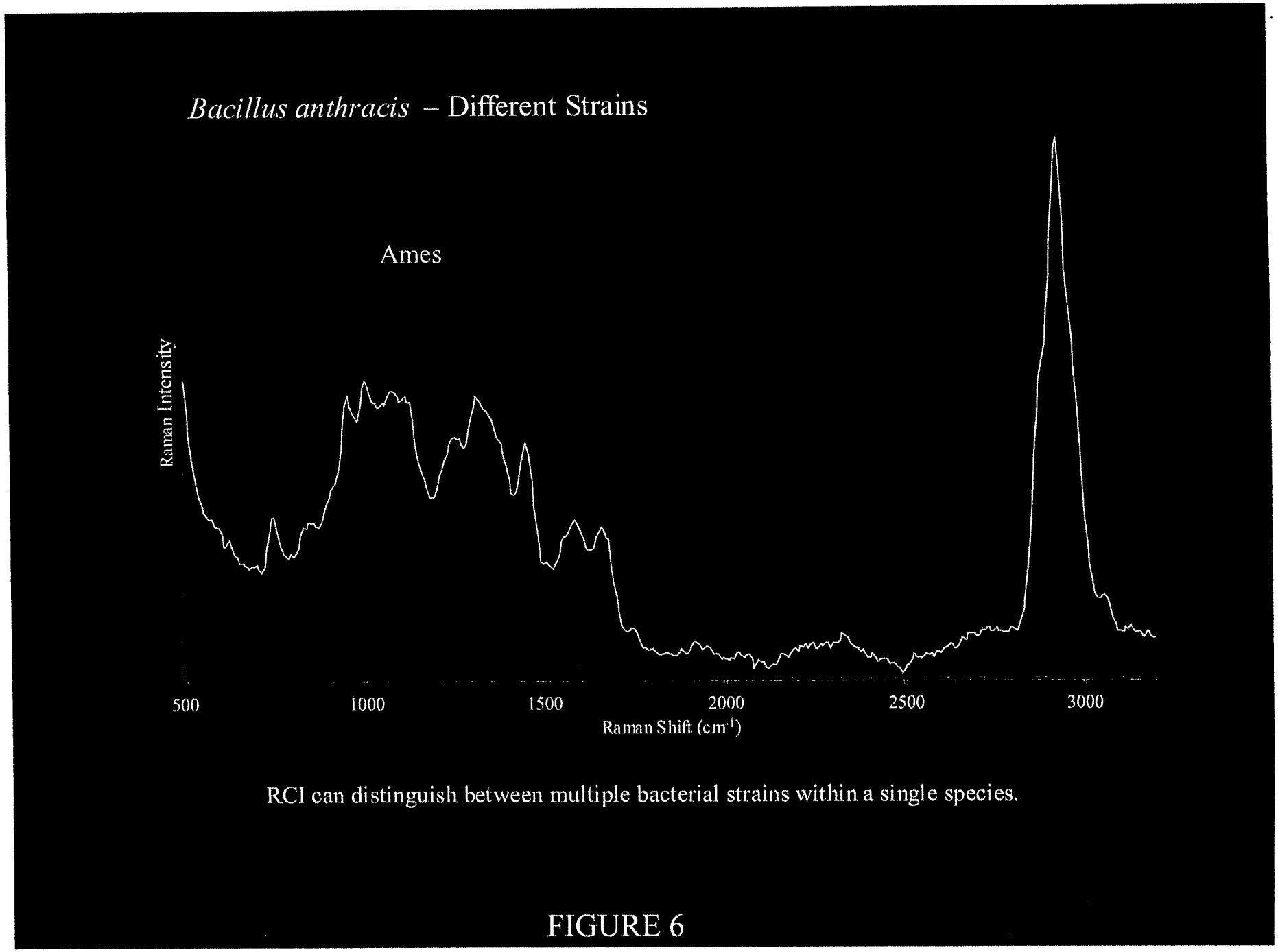
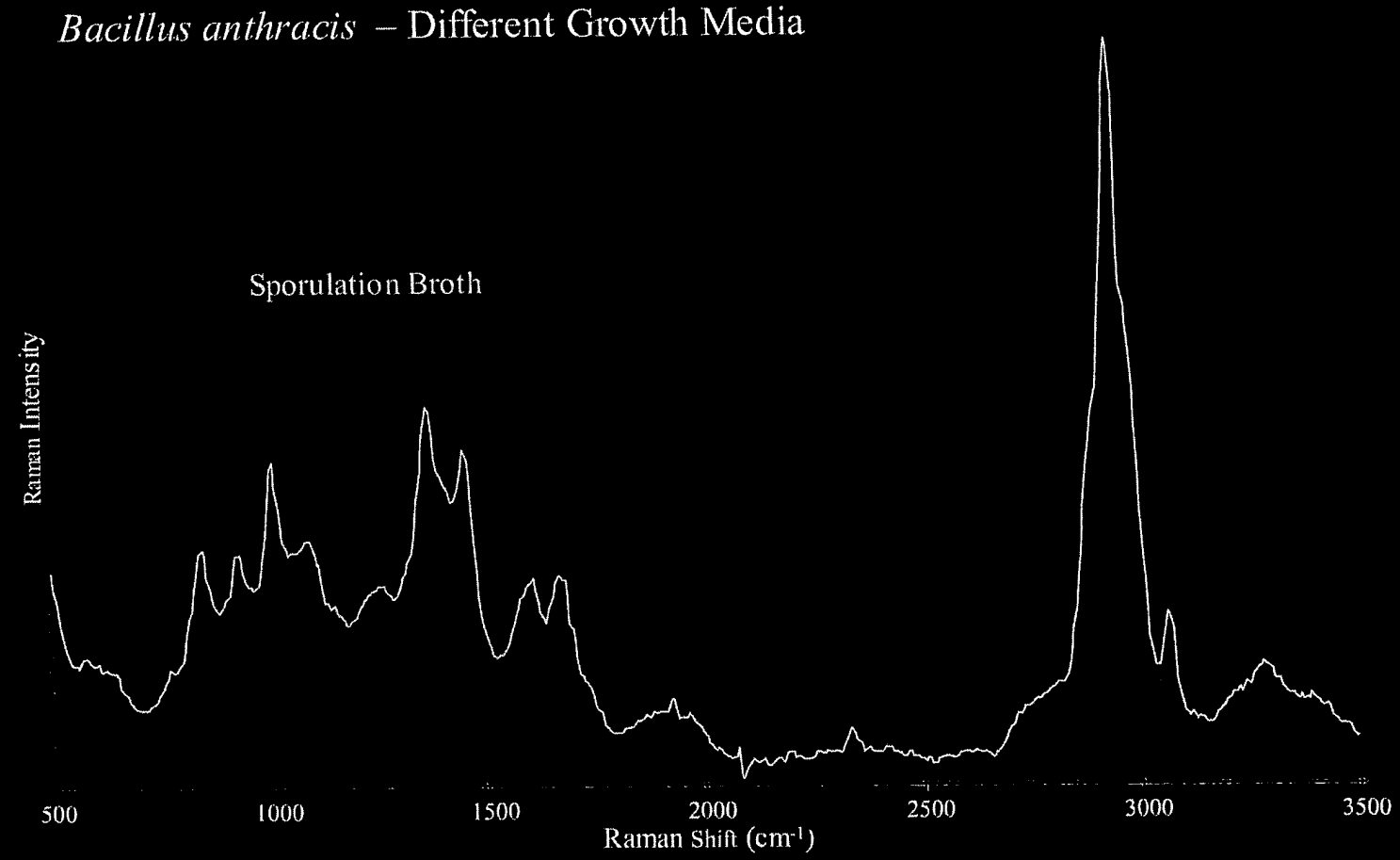

Dispersive Raman Spectra of AFIP Samples
Baseline-Corrected
A Bacillus thuringiensis
B Bacillus cereus
C Bacillus subtilis var. niger (BG)
Arbitrary Intensity
C
A
B
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
Raman Shift (cm-1)

Figure 5F



RCI can distinguish between multiple bacterial strains within a single species.

FIGURE 6



RCI can distinguish between different growth conditions.

FIGURE 7

Raman Spectra are reproducible

AFIP Samples – *B. Anthracis* in Sporulation Broth

Dispersive Raman Spectroscopy – 10 Different Regions of Interest

- Statistical Analysis (F-Test) indicates reproducibility to 95% confidence level
- Collected with FALCON Raman Chemical Imaging Microscope
- Data Acquisition Time: 60 sec/spectrum

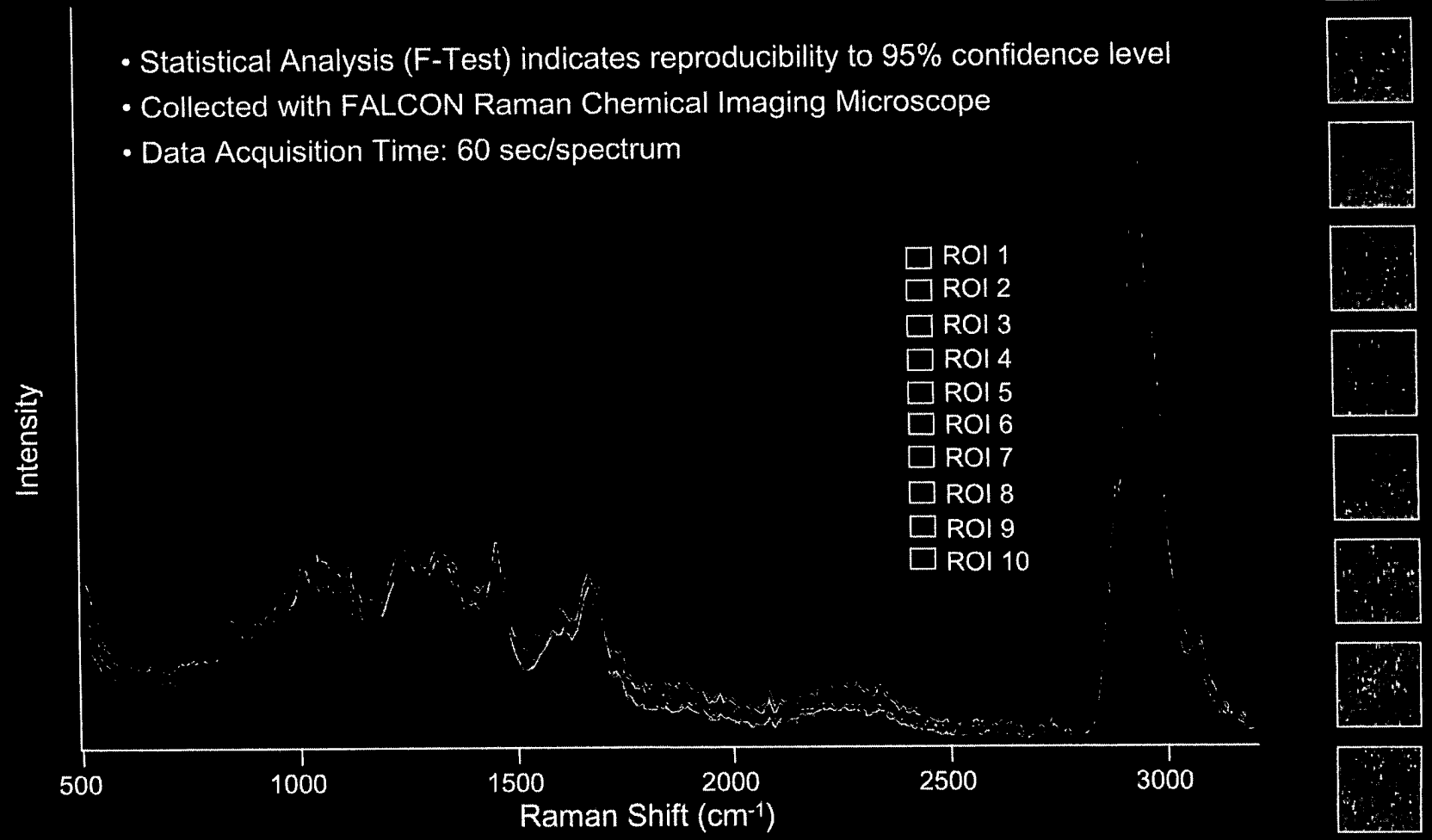


FIGURE 8

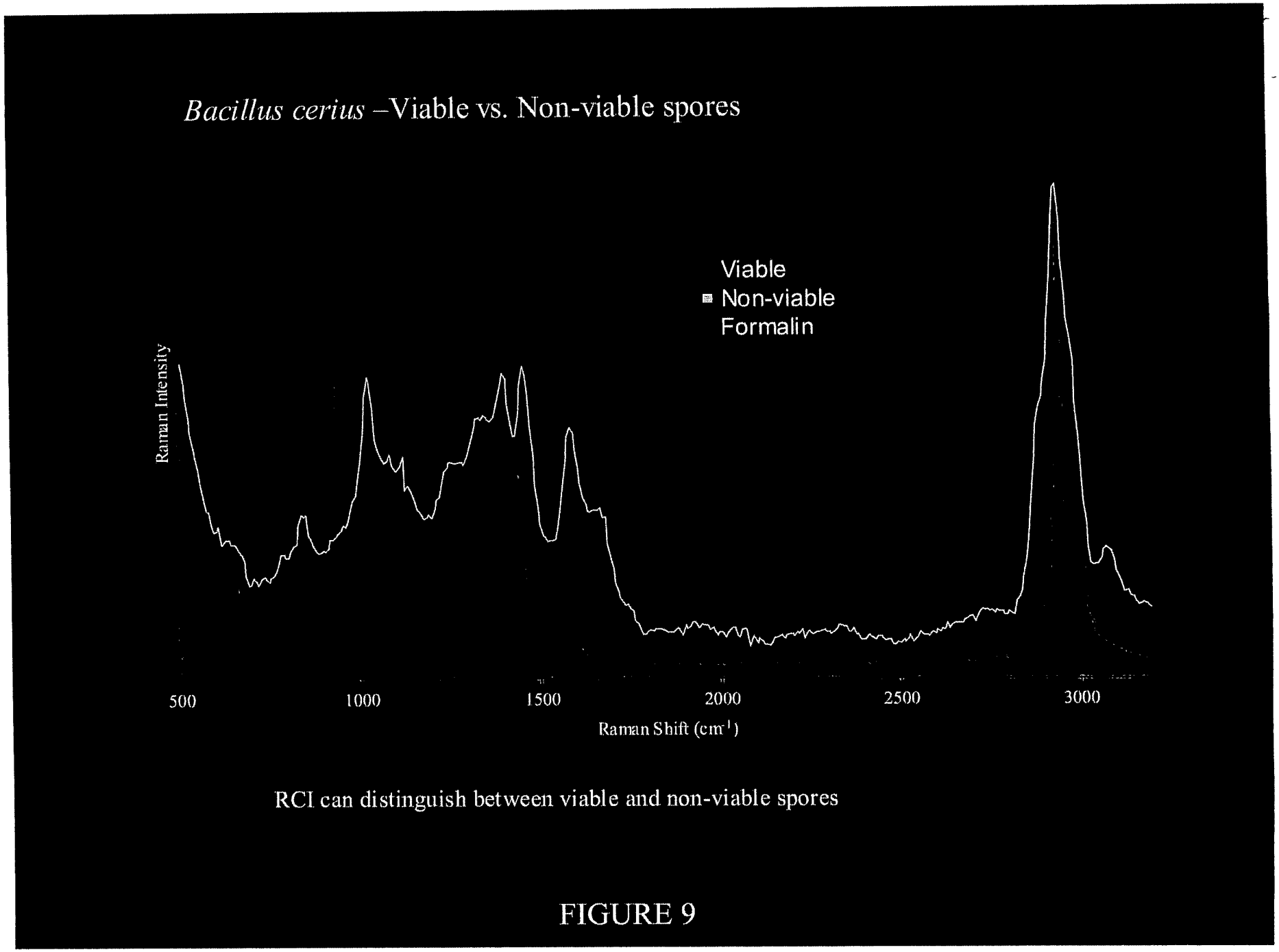

Bacillus cerius –Viable vs. Non-viable spores
Viable
Non-viable
Formalin
Raman Intensity
500
1000
1500
2000
2500
3000
Raman Shift (cm-1)
RCI can distinguish between viable and non-viable spores

FIGURE 9

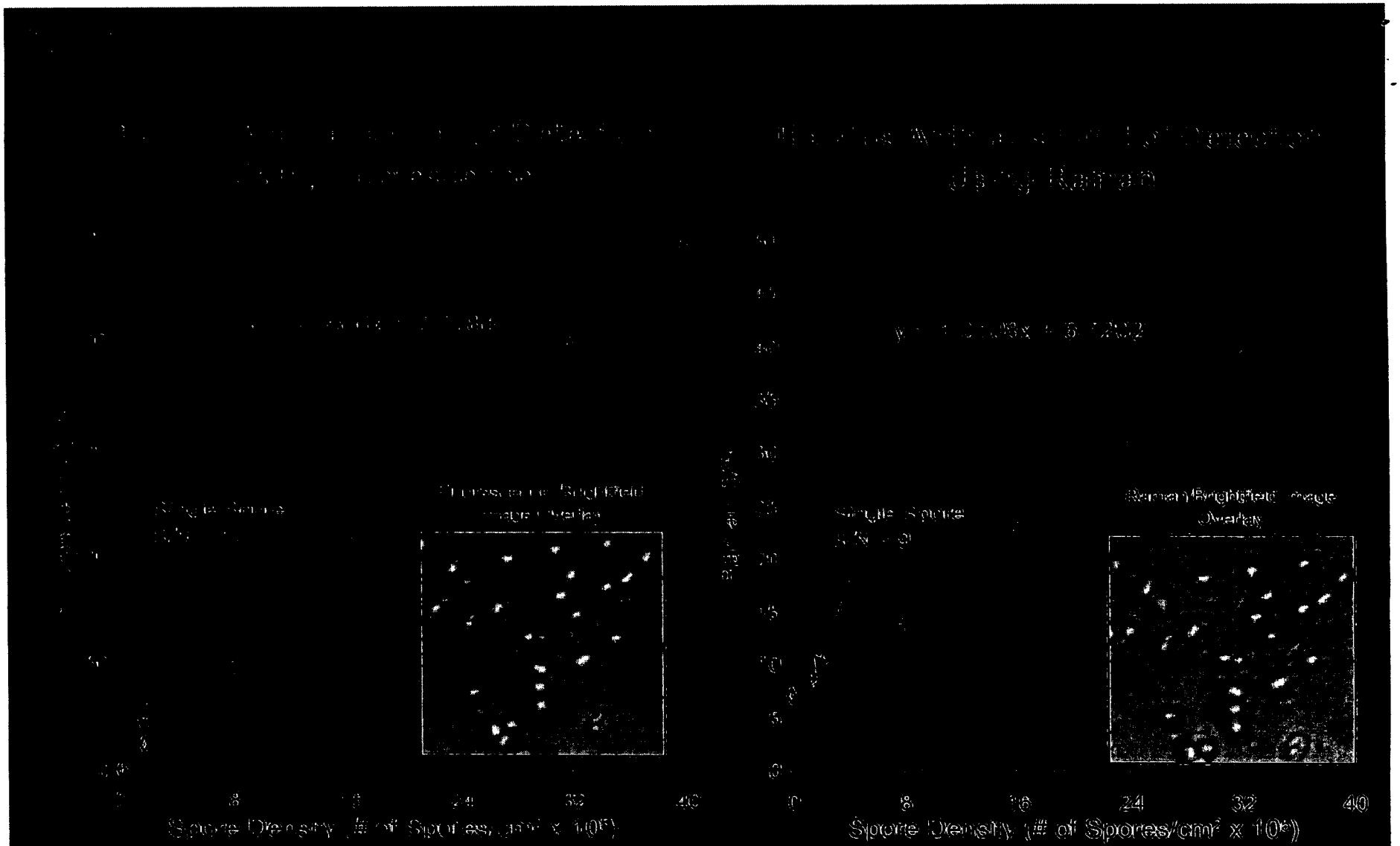

Spore Density (# of Spores/cm² x 10⁶)
Spore Density (# of Spores/cm² x 10⁶)

FIGURE 10

Preliminary Receiver Operator Characteristic (ROC) Curve
Bacillus anthracis Discrimination Assessment

- Using un-optimized discrimination approach, at 90% probability of detection, 5X improvement in false alarm rate demonstrated

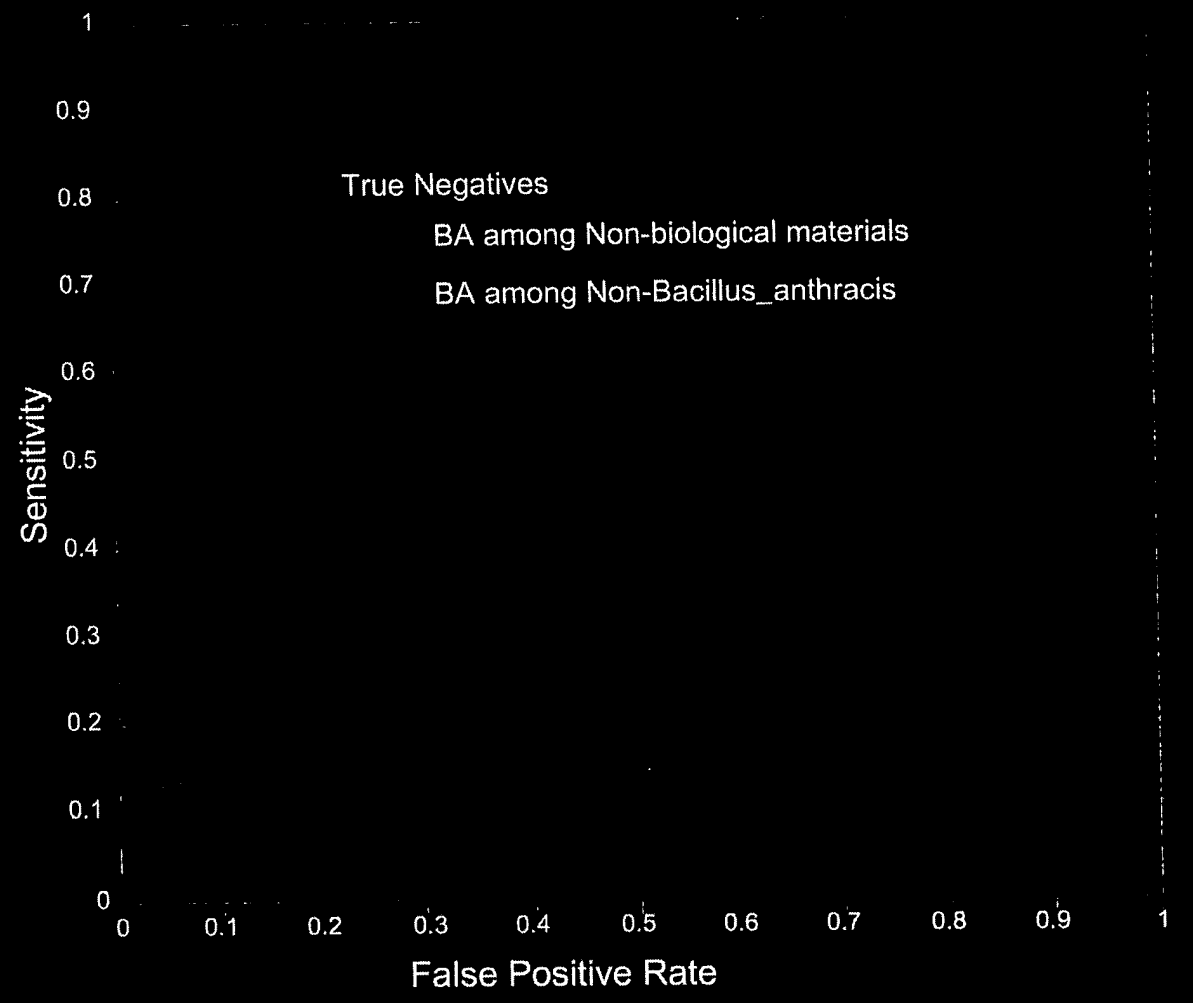
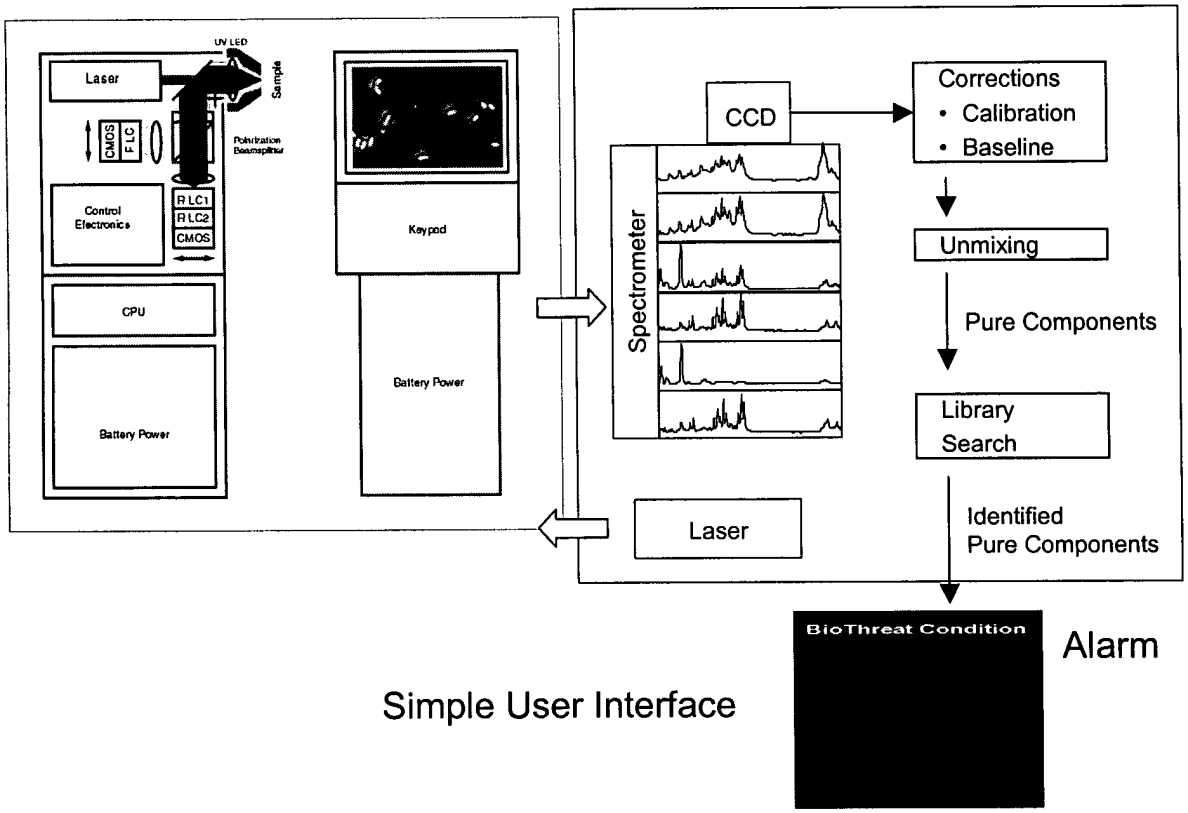


FIGURE 11

Handheld Pathogenic Microorganism Detector



- Handheld detection and identification system
- Reagentless point-detection system
- Fluorescence (screening) and Raman (diagnostic) Detection Capabilities
- Eye-safe, solar blind daytime operation through shielding at the probe tip
- Suitable for assessment of complex mixtures

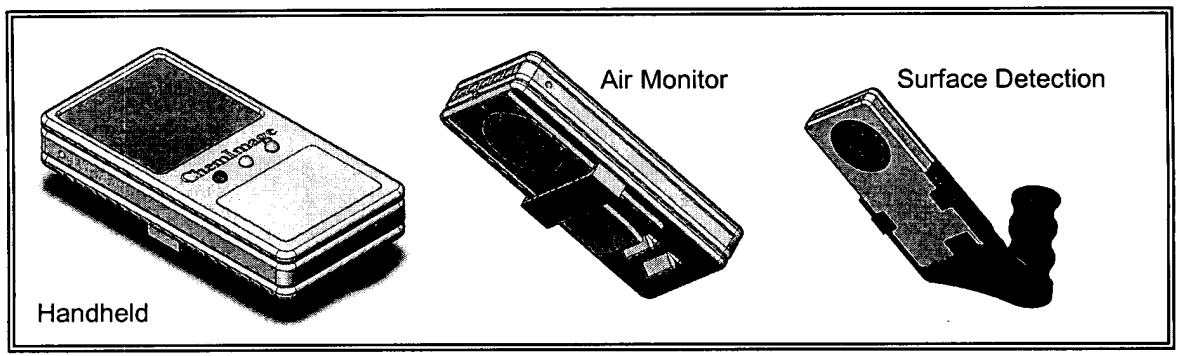


FIGURE 12